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### ABSTRACT

It has been reported by earlier workers in this laboratory (Bedford College) that there is a wide variation in the degree of stimulation by IAA of the growth of pea root sections. It was then suspected that effects of age, diffuse light in which sections were weighed and metallic impurities present in distilled water might modify this stimulation. Experiments were carried out to investigate the effects of these factors. The results of the experiments showed that age of the seedlings has a pronounced effect in the growth of root sections, but it does not alter the stimulatory effect of IAA.

Strong light promotes the growth of root sections. It also causes a shift in the optimal concentration of IAA from one part in  $10^{11}$  to one part in  $10^{10}$ . This seems to be due to IAA destruction.

Metallic impurities such as  $\text{Ca}^{++}$ ,  $\text{Mn}^{++}$ ,  $\text{Zn}^{++}$ ,  $\text{Cu}^{++}$  and Boron in dilute concentrations do not alter the response to IAA. Cobalt at  $10^{-5}\text{M}$  not only stimulates root growth but also antagonises both stimulatory and inhibitory effects of IAA.

It can finally be concluded that, since the concentrations of metallic impurities likely to exist in ordinary distilled water are much lower than any of the above concentrations of metals giving interference with IAA stimulation, variations in the quantities of metallic impurities in ordinary distilled water are not likely to be the cause of the previously observed variations in sensitivity to stimulatory concentrations of IAA.

The main concern of the investigations was studies on the interaction of IAA with antiauxins i.e. N.M.S.P., N.M.S.A., C.N.B. and P.C.I.B. in the stimulation of root section growth and also the interaction between antiauxins themselves. The investigations of this nature should throw some light on the hypothesis postulated by Audus and Shipton (1952) that auxin (IAA) and antiauxins stimulate the growth of root sections by antagonising the action of a natural inhibitor which holds the root growth below the possible maximum.

The results of the interaction experiments with these compounds at growth stimulatory concentrations support the hypothesis that both types of compound are exerting fundamentally the same action in the growth system but suggest that the action is a direct one and not due to an antagonism of a natural endogenous growth inhibitor.

### ACKNOWLEDGMENT

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My thanks are also due to Professors Audus, Arne Fredga, R. L. Wain, Dr. Børge Åberg, and Pal Chemicals Ltd. for their kind co-operation in placing at my disposal the chemicals used in conducting my experiments.

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## APPENDIX

All the original data of growth and interactions are presented in this appendix.



## CHAPTER I

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### INTRODUCTION

#### (a) Early work on root hormone

The foundation of our knowledge of plant hormones was laid by Charles Darwin as early as 1880 in his epoch-making discoveries of the reaction of plant organs to external stimuli such as light and gravitation. It is through his work that the phenomenon of geotropism was first known to plant physiologists. He first demonstrated that roots perceive the stimulus of gravitation at the tip. Decapitation of the root tip completely eliminates the response of root to this stimulus and he further showed that the reacting zone both in the coleoptile and root is located in the region of extension growth some distance behind the tip.

In contrast with the coleoptile, the decapitation of root tips results in a slight acceleration of growth (Cholodny 1924). But reheading the root tips on corresponding root stump produced further inhibition. He suggested therefore that possibly a growth-retarding hormone is produced in root tips. It was later discovered that both root and coleoptile tips when placed on decapitated roots cause retardation of growth suggesting that the hormone produced in both the root and coleoptile is the same.

The production of auxin in root tips was demonstrated by Hawker in (1932) by placing the root tips of Vicia Faba on gelatin. Boysen-Jensen (1933) confirmed Hawker's finding using dextrose agar. Thimann (1935) using direct method of extraction with chloroform was able to extract auxin from Avena root tip.

The hormone that diffuses from root tip is probably B-indole acetic acid, and in the range of concentrations found in shoot or coleoptile, it always retardsthe growth of root (Boysen-Jensen, 1928).

(b) Role of auxin in the extension growth of root

In the development of our knowledge of plant hormones and their functions, the relation of auxins to the growth of root is very little understood.

It has been clearly shown (Thimann, (1936), Lane, (1936), Bonner and Koepfli (1939)) that while the elongation of coleoptile and stem is promoted, the elongation of root is inhibited by the application of auxins from without. These two seemingly diverse manifestations of the effects of externally added auxins attracted the increasing attention of plant physiologists and considerable experimentations have been done in recent years in an attempt to elucidate precisely the mechanism by which growth regulators control growth in these two different tissues.

Nielsen (1930) first discovered the inhibitory effect of crude extract of *Rhizopus* culture medium on the growth of root. Kogl, Haagen-Smit and Erxleben (1934) similarly showed that pure substance such as, auxin a, auxin b and indole-acetic acid caused retardation of root growth. Indene-3-acetic acid which possesses the properties of all auxins, also depresses the growth of root. This observation led Thimann (1935<sup>a</sup> 1936) to suspect that an inhibition of root growth by growth regulators might be one of the general properties of all auxins.

Bonner and Koepfli (1939) investigated the activity of as many as 21 homologues of IAA on the growth of *Avena* root. They found that some compounds such as, N.A.A., IAA, and cis-cinnamic acid, which caused promotion of the growth of coleoptile, were also highly active in inhibiting the root growth. On the other hand some compounds such as cyclohexane-acetic acid and trans-cinnamic acid, which were less active in promoting stem elongation, were also less effective in inhibiting the growth of root.

They, therefore, reached the conclusion that the chemical structure which a molecule must possess in order to exert growth activity in stem or coleoptile is closely similar to that which it must possess in order to be effective in root inhibition.

(c) Stimulation of the growth of root by IAA

In contrast to the inhibition, extremely low concentrations of auxin cause a small but definite acceleration of root elongation. Amlong (1936) decapitated Vicia roots and allowed them to stand for 3 hours to deplete their internal auxin supply. If it was then subsequently treated with  $10^{-9}$  Molar of IAA, the growth was accelerated. Fiedler (1936) found that when zea roots were grown in medium containing yeast extract; all concentrations of added auxin inhibited elongation, but when the yeast extract (which contained some auxin) was omitted, then  $2 \times 10^{-9}$  molar indole acetic acid accelerated growth by some 30%. With same material Geiger-Huber and Burlet (1936) independently found that the optimum concentration of indole-acetic acid was about  $3 \times 10^{-11}$  molar. At this concentration the stimulation caused was about 30% <sup>over the</sup> untreated control. Thimann reported (1936) that  $10^{-9}$  M. IAA caused about 30% stimulation. The response of the roots to auxin is given by optimum curve with its peak at extremely low auxin concentration. Recently Audus and Garrard (1953), Audus and Shipton (1952) reported that one part in  $10^{11}$  of IAA causes a stimulation of pea root sections of the order of 25%. This stimulation by low concentration of IAA is very well-marked in early hours. Concentration one part in  $10^{10}$  also produces stimulation about 8%.



Similar stimulation of the growth of lateral buds by very dilute concentration of auxin was observed by Thimann (1937). The apical buds which produce auxin suppress the growth of the lateral ones. This inhibition in lateral buds is caused due to the translocation of auxin produced in apical buds to lateral ones. The very low concentrations of auxin accelerate the growth of the root so also when very dilute concentration of auxin is treated to lateral buds, there can be seen an increase in weight of the lateral buds. This closer parallelism of auxin action in root and <sup>also</sup> in lateral buds gives added support to the hypothesis (Thimann 1937): that apparently different action of auxin on different organs depends upon their response to different concentration ranges. In principle, stem, roots, and buds behave in the same way. Thimann (1935 $\alpha$ ) postulated the theory that auxin enters into some 'master reaction' and that other properties of particular tissue then determine what sort of effect will be manifested.

Audus and Shipton (1952) postulated a theory that the stimulation of root growth, caused by very dilute concentrations of IAA and certain antiauxins, <sup>i.e.</sup> N.M.S.P., P.C.I.B., may be due to the antagonism of an endogenous natural inhibitor of the root which holds the growth of the root below maximum.

Auxins and antiauxins both in the same identical way possibly, push off the growth inhibitor from the inhibiting

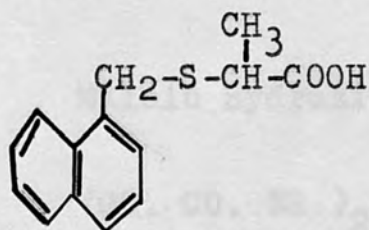
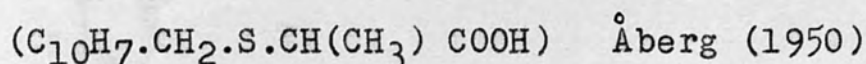
growth centre, thereby relieving the root from the inhibition. Finally this antagonism of inhibitor results in stimulation of root growth.

(d) Antiauxins and the growth of root

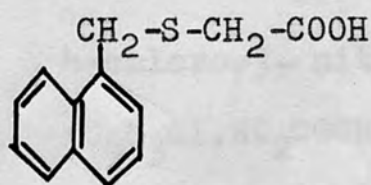
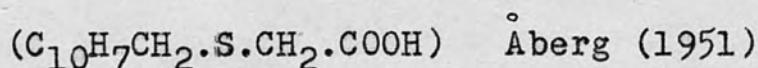
In recent years increasing attention has been paid to the study of the interaction of auxins and antiauxins in the control of root growth.

A few important antiauxins which are at present known are:-

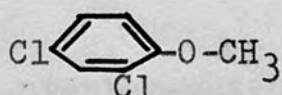
- 1) N.M.S.P.  $\alpha$ -(1-naphthyl-methyl-Sulphide) propionic acid.



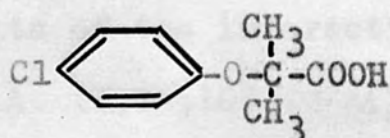
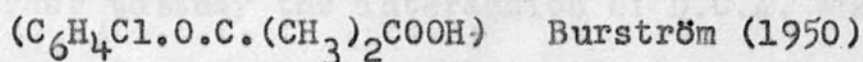
- 2) N.M.S.A. (1-naphthyl-methyl-Sulphide-acetic acid)



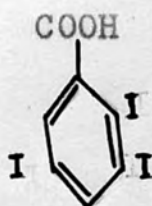
- 3) D.C.A. 2,4-dichloroanisole



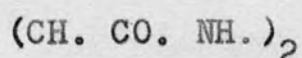
- 4) P.C.I.B.  $\alpha$ -(p-chloro-phenoxy)-iso-butyric acid



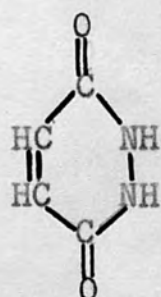
- 5) T.I.B.A. 2,3,5,-triiodo-benzoic acid



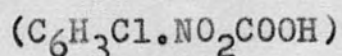
- 6) M.H. Maleic Hydrazide, more properly 1,2,dihydro-pyridazine-3,6 dine.



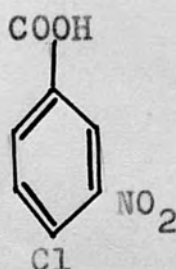
Leopold and Klein (1951)



- 7) C.N.B. 4-chloro,3-, nitro-benzoic acid



Minarik et al. (1952)



Activity of 2,4-Dichlor-anisole.

Audus and Shipton (1952) conducted a series of experiments



with D.C.A. in order to study the interaction of D.C.A. and 2,4-D in the growth of cress root seedlings. An analysis of results of the interaction between 2,4-D (.01, 0.1, 1 (P.P.M)) and D.C.A. (1, 10, 100 (P.P.M)) showed that there was no interaction between D.C.A. and 2,4-D. The study of the interaction

between D.C.A. (.01, .02 and 0.1) and M.C.P.A. (1, 10 ppm) also did not show any interaction. Experiments conducted with D.C.A. (.01 and 1 ppm) in combination with 2,4-D  $10^{-5}$ ,  $10^{-4}$  ppm) on the growth of excised pea roots also did not indicate any interaction. These results go against that reported by Mc. Rae and Bonner (1953) who showed a marked interaction between these two compounds in *Avena* coleoptile. What causes this difference in activity in root and coleoptile is difficult to say. Possibly D.C.A. may be a weak antiauxin in the growth of root.

2,3,5 <sup>6</sup>Tri-iodobenzoic acid: (T.I.B.A.). The augmentation of flowering, loss of apical dominance, shortening of internodes, in Soya bean may suggest that T.I.B.A. is an anti-auxin, if it were not the fact that its low concentration ( $10^{-6}$  molar) applied to split pea internodes increases the activity of applied auxin <sup>(IAA)</sup> as shown by Thimann and Bonner (1948).

Galston (1947) suggested that effects of T.I.B.A. might be mediated by auxin because it could considerably antagonise the effect of IAA in Avena curvature test present in the proportion of 5:1. In much higher concentrations it completely nullified the effect of IAA.

Thimann and Bonner (1948) found that at concentrations ( $5 \times 10^{-5}$  and  $10^{-7}$  M) T.I.B.A. alone caused curvature of split pea internodes. This positive effect disappeared when the slit sections were washed for 2 hours in water before the

experiment. This result suggests that T.I.B.A. effect is mediated by residual auxin system in stem. Low concentrations (25-100 mg/litre) used in combination with IAA and 2,4-D induce much greater curvature in slit pea internodes (synergistic effect).

Aberg's experiments with T.I.B.A. (1953) on the growth of flax root revealed that a range of concentration of T.I.B.A. ( $10^{-8}$  -  $10^{-6}$  M) caused first decline of the action curve, which, according to him, is a synergistic effect upon native auxin. At somewhat higher concentrations ( $10^{-6}$  -  $10^{-5}$  M) the action curve remains at the same level but at concentration higher than that i.e. ( $10^{-5}$  -  $10^{-4}$  M) there is a sharp decline of the action curve.

This inhibition is due to toxicity on the protoplasm of the root cells and is not related to auxin.

When a low concentration ( $10^{-6}$  M) T.I.B.A. was used with low concentration of IAA - a synergistic effect appeared. No such synergistic effect was detected with low concentration of 2,4-D.

Fairly high concentration  $3 \times 10^{-5}$  M of T.I.B.A. antagonised the inhibition caused by IAA or 2,4-D.

The superposition of inhibition brought about by small amount of T.I.B.A. and 2,4-D led him to suggest that T.I.B.A. possibly increases the native auxin IAA inside the root. This increment of the activity of native auxin is possibly added to the direct effect of 2,4-D. He assumed



that IAA is the principal native auxin and T.I.B.A. exerts its influence on the enzyme system regulating the IAA metabolism in plant cells. T.I.B.A. possibly inhibits the effect of IAA oxidase system.

Maleic hydrazide:- Maleic hydrazide was then tried by Åberg (1953) on the growth of flax root. It was found that maleic hydrazide at any concentration, however dilute it was, stimulated root growth. In this way M.H. differs with T.I.B.A. which stimulates root growth in dilute concentration, i.e. ( $10^{-6}$ M). At higher concentration ( $10^{-4}$ M) of M.H., there was marked inhibition which was due to toxic effect upon the root cells. These results of Åberg agree well with those reported by Leopold and Klein (1951, 1952) who also obtained a conspicuous inhibition of pea stem at  $10^{-6}$ M. When M.H. was used in combination with IAA in flax root, an antagonism between them was observed. (Åberg 1953).

He, therefore, suggested that an antiauxin effect of M.H. is perhaps caused by an accelerating effect upon IAA - oxidase system i.e., M.H. accelerates auxin destruction in root.

In strong contrast with the antiauxins mentioned above, certain antiauxins cause stimulation of root growth at moderately high concentrations. These antiauxins are;- N.M.S.P, and its homologues, P.C.I.B. and its homologues; and C.N.B. (4-chloro-3, nitro-benzoic acid) and its homologues. This stimulation of root growth by antiauxins is attributed

to their antagonistic effect on the native auxin of root which is supposed to be in supra-optimal concentration. (Boysen and Jensen 1928). These antiauxins possibly reduce the supra-optimal concentration to optimal one and thereby cause promotion of root growth. From many reasons (see discussion pp 116 ) this idea of root to have supra-optimal concentration sounds doubtful.

P.C.I.B.:- It was Burström who first (1950) discovered that certain isobutyric compounds such as P-chlorophenoxy-isobutyric acid, (P.C.I.B.) indole-3- isobutyric acid and their homologues stimulated the growth of intact root of wheat seedlings. The magnitude of stimulation by ( $10^{-6}$  and  $3 \times 10^{-6}$  Molar) concentrations of P.C.I.B. and indole-3-isobutyric acid is about 60% in first two days but slightly less up to sixth day. A range of concentration ( $3 \times 10^{-7}$  -  $3 \times 10^{-6}$  M) always accelerated the growth of the wheat root. Roots treated with P.C.I.B. completely lacked root hairs; but there was usual consequence of an increase in cell elongation.

He envisaged that P.C.I.B. has an effect on cell elongation opposite to the effect of IAA. IAA was shown to accelerate the first phase of cell elongation and shorten the second phase, while P.C.I.B. slows down the first phase and accelerates the second phase.

P.C.I.B. effects the root growth in the following way:

- 1) It retards the first phase of cell elongation.
- 2) It accelerates the second phase, this action dominates and results in the greatly increased gross length of the roots.
- 3) Partly but not wholly as a consequence hereof the root hairs disappear and the development of lateral roots slows down.
- 4) In higher concentration it retards the cell multiplication, perhaps a slight specific effect, distinct from real growth action.

In every respect, except for the last one, P.C.I.B. effect is just the reverse of the action of auxin on roots.

He then studied the effect of P.C.I.B. in combination with IAA. When ( $10^{-8}M$ ) concentration of IAA was used in combination with ( $10^{-6}M$ ) of P.C.I.B. IAA reduced the cell length much less than that of P.C.I.B. alone. P.C.I.B. at this concentration also counteracted the inhibition caused by  $10^{-6}M$  of IAA. These results suggest that P.C.I.B. really acts as an antiauxin to IAA. Burström is of the opinion that P.C.I.B. possibly blocks the native auxin from its place of action.

His subsequent experiments with different isobutyric derivatives brought to light the following results.

2 or 3-monochloro, 2,4-dichloro, 2,4,5-trichloro, and 2,3,4,5,6-pentachloro, phenoxy-isobutyric acids are also



active to a lesser degree in stimulating the root growth.

Para chlorinated isobutyric acid is more active than any of the ortho; meta derivatives. This means that only substitution in para-position decidedly increases the activities of the isobutyric acids.

N.M.S.P. :- N.M.S.P. and its homologues were tried by Åberg in (1950,1951) the growth of flax roots.

1-N.M.S.P. alone causes stimulation of the root growth at a concentration range ( $10^{-5}$ - $10^{-6}$ M) showing an optimal stimulation of about 15-20% at concentration  $10^{-5}$ M. At low concentration ( $10^{-8}$ M) there is no detectable stimulation.  $10^{-4}$ M causes a marked inhibition not related to auxin. The naphthyl-methyl-selenide acids differ somewhat from sulphur compounds, having a wider stimulation range with conspicuous effect even at  $10^{-8}$ M. The degree of optimum stimulation at  $10^{-6}$  is about 25%.

When a range of concentrations ( $10^{-8}$  to  $2 \times 10^{-5}$ M) of N.M.S.P. was used in combination with ( $10^{-7}$ M) of 2,4-D, it was observed by Åberg (1950,1951) that N.M.S.P. alleviated the inhibition caused by 2,4-D <sup>alone</sup>. This restorative effect of N.M.S.P. upon root growth was well-marked at  $2 \times 10^{-5}$  M. of N.M.S.P. He suggested that the restorative effect of N.M.S.P. upon root growth varies with the degree of inhibition caused by growth substance alone probably passes a maximum value at medium inhibition.



Certain homologues of N.M.S.P., such as N.M.S.A., N.M.Se.A. (selenide) compounds at appropriate concentrations ( $10^{-5}$ - $10^{-8}$  M) also alleviate this 2,4-D inhibition of root growth.

Further experiments on the interaction of N.M.S.P. and IAA or N.A.A. showed that these auxins antagonise the stimulation by N.M.S.P. alone.

On the basis of above results Åberg suggested that the antagonistic action of antiauxins depends upon a competitive displacement of auxin 2,4-D from the growth centre, and there is a connection between the adsorbed amount of antagonists and displaced amount of 2,4-D. It is proportional to  $(1-A)$  where A is the amount of 2,4-D displaced, from the growth centre. He assumed that antiauxins which have a low inhibiting activity displace the highly active auxins at this inhibiting centre, thus partially relieve the inhibition of root caused by auxins.

He postulated two molecular properties relevant to these effects :

- (a) The "affinity" of the molecule for the growth centre.
- (b) The "activity" (inhibition in root) of the molecule when attached to this centre.

It is therefore suggested that antiauxins have high "affinity" but low "activity". If this assumption is true then weak auxins having low activity will also antagonise strong ones.

4-Chloro-3-nitrobenzoic acid (C.N.B.)

Among 35 substituted benzoic acid tested by Minarik et al. (1952) on the growth of cucumber roots, 4-fluoro, 3-nitro-benzoic acid (F.N.B.A.) and other substituted 3-nitro, 4-halogen-compounds stimulated the root growth. The stimulation of fluoro compound occurred at 10-40 p.p.m. was about 80% over the untreated controls. They suggested that this stimulation was caused by an antagonistic effect of a naturally occurring auxin which is in supra - optimal concentration.

In their subsequent experiments on the interaction of 2,4-D (0.01, .1, 1.0 p.p.m.) and F.N.B.A. (0.1, 1, 10 p.p.m.) it was discovered that F.N.B.A. alleviated the 2,4-D inhibition of cucumber roots at a ratio of this compound to 2,4-D of 10:1

(e) Statement of the Problem

All these reports suggest that there is a clear-cut antagonism between auxins and antiauxins in the inhibition of root growth. But no investigation hitherto has been pursued on the interaction of auxins and antiauxins in the stimulation of root growth. Studies on the interaction between stimulatory concentrations of IAA (B-indolylacetic acid) with a range of stimulatory concentrations of antiauxins, such as N.M.S.P., C.N.B., P.C.I.B. may throw light on the mechanism by which these auxins and antiauxins stimulate the

growth of root. In this thesis results of the experiments on the interaction of low concentrations of auxins (one part in  $10^{11}$  and one part in  $10^{10}$ ) and allied compounds will be presented.

(f) Statement of immediate problems

But before the investigation on the interaction of auxin and antiauxins was undertaken, studies on some other problems needed immediate attention and careful experimentation. These are, the effects of age, of light and of metallic impurities to IAA stimulation of the growth of pea root sections. So experiments on these immediate problems were first carried out.

The earlier workers in this laboratory (Bedford College) reported that a wide variation in the stimulatory effects of dilute concentrations (one part in  $10^{11}$  and  $10^{10}$ ) of IAA on the growth of root sections did occur, but the causes of this variation were unknown.

It was then thought that effect of age of the seedlings, effect of diffuse sun light in which sections were cut and weighed, and also the presence of metallic impurities in ordinary distilled water might modify IAA stimulation of the root sections. Experiments designed to study the effect of these factors were first carried out. The results will be shown in this thesis.

## Introduction

It had been reported by Galston and Hand (1949), and Galston and Baker (1949) that the growth of etiolated stem sections in light is much less than in dark. Inactivation of added IAA took place in light. This report made me feel that possibly diffuse light might affect this variation by changing the sensitivity of root sections to added IAA or light might cause the inactivation of IAA. Nobody had ever tried to find out this effect of light on the stimulation by IAA.

Metallic impurities in the medium greatly alter the growth of tomato root sections. This was clearly shown by Boll and Street (1951). They first discovered that the growth of tomato root sections in White's medium prepared at Manchester was poor, while that in medium prepared at Nottingham was high. They then investigated the factors responsible for the poor growth of excised tomato roots encountered in certain batches of White's medium. They found that this variation was due to metallic contamination in ordinary distilled water. Addition of copper as copper-sulphate, of molybdenum as molybdic acid to the medium resulted in an improvement of the growth of sections.

It was, therefore, considered necessary to investigate the effect of metallic impurities on the stimulatory effect of IAA before the investigation on the effect of auxin and antiauxins on the stimulation of root growth was carried out.



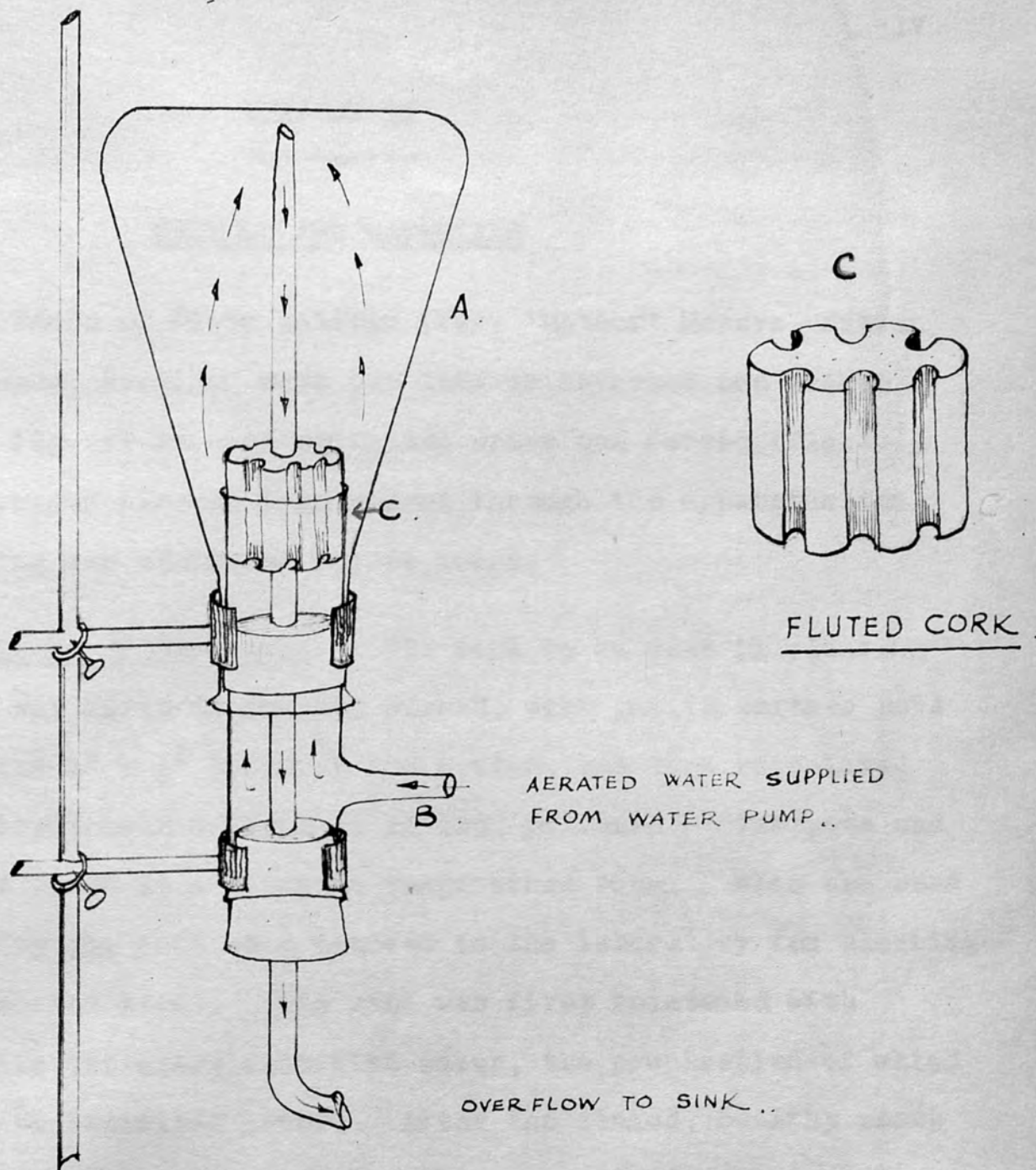


Figure I

Diagram of the inverted pea soaker.

## CHAPTER II

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### METHODS AND MATERIALS

Seeds of *Pisum sativum* (var. 'Meteor' Messrs. Sutton and Sons, Reading) were put into an inverted pea soaker (See Fig. I) through which tap water was forced (Fig. I. ) Compressed air was then passed through the apparatus and soaking was continued for 24 hours.

- (a) Culture of Seedlings: The sand to be used in planting peas was first thoroughly washed, then put in earthen pots of size 8" x 4" bored at the bottom, and then autoclaved for about half an hour at 15 lbs. pressure. The pots and sands dried in a constant temperature room. When the sand was dry the pots were removed to the laboratory for planting the soaked seeds. The sand was first moistened with sterile tri-glass distilled water, the preparation of which will be described later. After the soaked, healthy seeds were planted in these pots, they were placed on aluminium trays containing a little glass-distilled water. The pots were covered with glass tops to prevent the planted seeds from drying out quickly. They were transferred to the constant temperature room (temperature is about 25°C. and humidity is about 95%) and kept inside a cupboard so that the seeds germinate in complete darkness.

(b) Preparation of distilled water: Special tri-glass distilled water was prepared for use in the experiments. This tri-glass distilled water is free from all metallic impurities.

Process of distillation: Ordinary domestic tap water was distilled from a Manesty glass still. This ordinary distilled water was then run through an ion-exchange resin column called Biodeminrolit, supplied by Permutit and Co., to eliminate all metallic ions. The speciality of the Biodeminrolit resin is such that it adsorbs both cations and anions. This distilled water free from ionic impurities was again double distilled by passing through Bara-glass still. The tri-glass distilled water, thus prepared, was collected and used in experiments, making dilutions and preparing  $\frac{1}{2}\%$  sucrose solutions.

Audus and Garrard (1953) showed that 2·m.m. long root sections excised from the extending region of the radicle 1·0 - 1·5 m.m. below the extreme tips gave optimal growth compared with any similar sections cut from any other region of the radicles and subsequently grown in  $\frac{1}{2}\%$  sucrose solution. Therefore, in all the experiments described in this thesis 2 m.m. long sections were cut from 1· - 1·5 m.m. behind the tip.

Prior to excision, 2-day old, healthy and straight radicles of equal length were selected and taken out as a



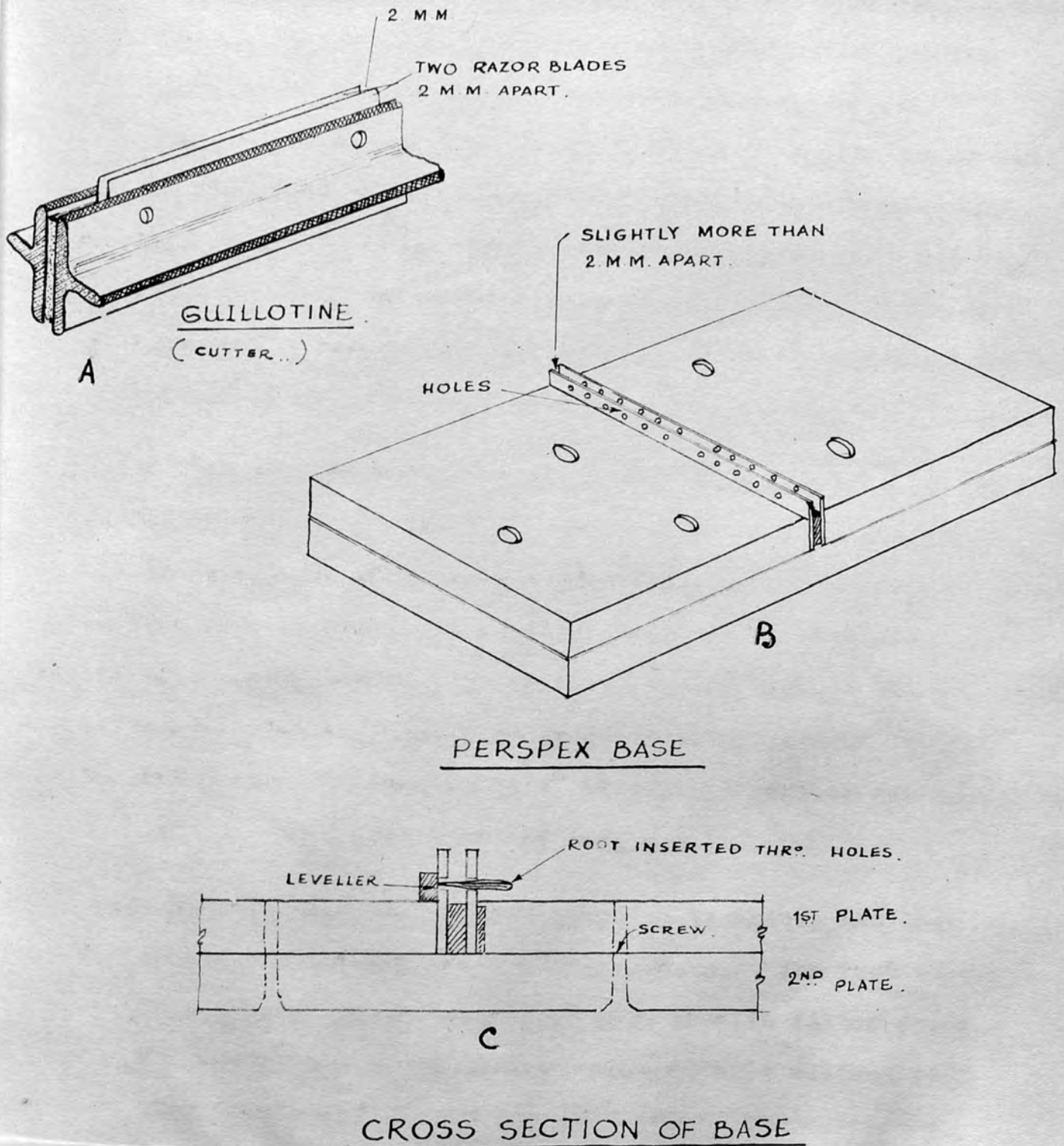


Figure 2

Diagram of the guillotine, base and the cross section of the base.

whole and were washed thoroughly in tri-glass distilled water. The washed radicles were then cut by means of a special guillotine, the sketch of which is shown in Fig. 2

The guillotine is a double bladed cutter consisting of two Duplex razor blades which are clamped in a holder nearly 2 m.m. apart.<sup>(Fig. 2A)</sup> The base of the cutter, (Fig. 2B), has two parallel uprights with 12 bored holes, through which <sup>were</sup> ~~are~~ inserted the radicles to be cut. These two uprights are slightly more than two m.m. wide.

(c) Cutting technique: The cutting technique is a slight modification of that used by Brown and Sutcliffe (1950). The radicles to be excised were inserted through holes in the base (shown in Fig. 2c) on one side and stretched across the channel between two uprights in the base. The apices of the radicles were then levelled by a leveller. The cutter was then pushed into the channel between two uprights. In this way 2-m.m. sections were cut.

(d) Experimental procedure: Sampling technique had been adopted throughout all the experiments. The root sections were pooled and surface-dried with sterile filter paper. Random samples of 10 sections were quickly weighed at a time to the nearest 0.1 mg. on a micro-torsion balance. Ten sections constitute a sample. The weighed sections were then transferred to a filter paper bridge dipping into

Figure 3

Diagram showing the method of growing excised segments from the extension zone of parent pea roots on  $\frac{1}{2}\%$  sucrose solutions under conditions of maximum aeration. (By kind permission of Professor L.J. Audus.)

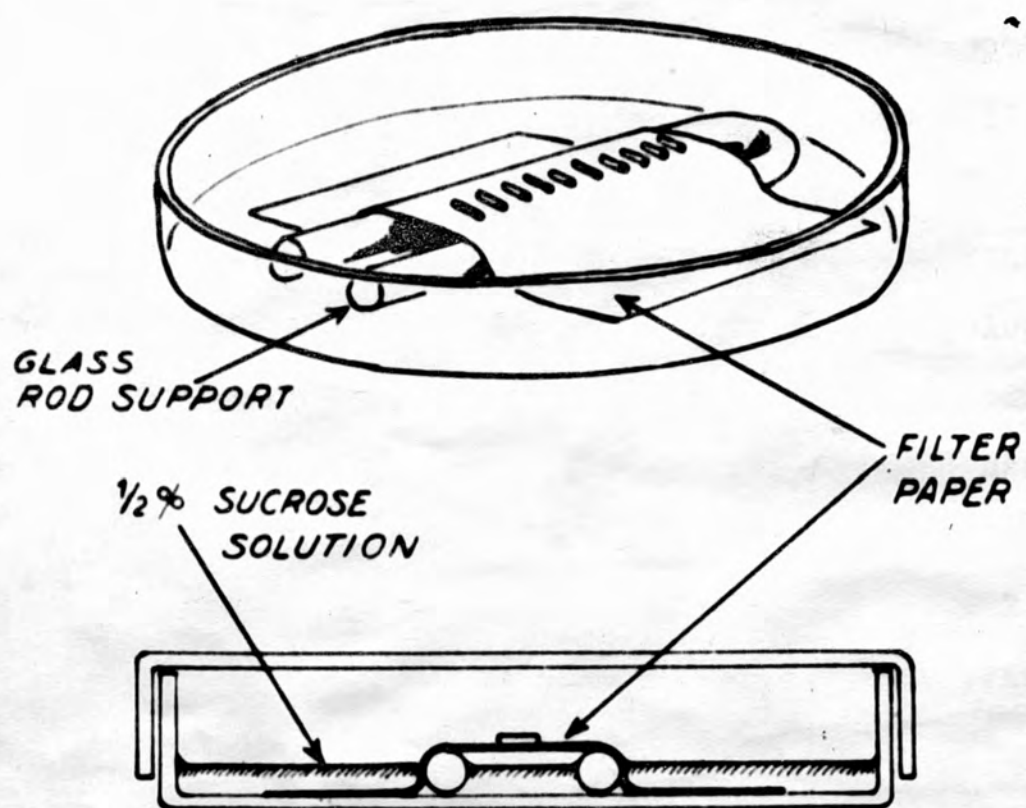


Figure 3



the test solution and an entry as a wick for its supply to the sections (See Fig. 3). The whole device was made to ensure maximum aeration which is necessary to obtain optimal growth. The sections placed on the filter paper were then covered with a lid. The petri dishes containing sections were kept in incubator at 25°C. and humidity of about 95%.

(e) Growth measurement:

(I) Weighing of sections:

The weighing of sections on a torsion balance was introduced by Audus and Shipton (1952). This is a much quicker technique than measuring the length of the sections. The whole process of drying and weighing of the sections can be performed in  $\frac{1}{2}$  minute. This technique was used with the supposition that there is no increase in thickness of the sections and the increase in fresh weight is due to extension along the main axis.

The growing of sections in filter paper was abandoned because of the fact that the stimulation caused by one part in  $10^{11}$  and also one part in  $10^{10}$  is much smaller than that obtained by Audus and Garrard (1953).

The stimulation they obtained was of the order of 25% with one part in  $10^{11}$  of IAA. So also the magnitude of inhibition by one part in  $10^8$  IAA is much smaller than that reported by them. This loss of stimulation and decrease of inhibition may be possibly due to inactivation of IAA by filter paper.

In later experiments the sections were immersed in the test solutions contained in the sterile petri dishes. During the growth period the petri dishes containing the growing sections were rocked through an angle of  $45^{\circ}$  from the horizontal on an electrically-driven device. Such agitation has previously been shown to be necessary to obtain optimal growth (Audus and Garrard, 1953).

(II) Length measurement: With the lid on, a petri dish was raised slightly on one side by means of a support. The sections were then arranged horizontally and length was measured along the longitudinal direction with a travelling microscope to the nearest .05 m.m. The length of ten sections constituting one sample was measured at a time. After the measurement of the sections the petri dishes were returned to the agitator and the electric motor was again turned on. The length measurement was performed at the regular interval of (0-4), (4-7), (7-24) and (24-48) hours. The growth of sections was observed for 48 hour periods.

Both growth measurements give a direct measure of extension growth, since sections do not show any change in thickness during extension except in high auxin concentrations. The first method has the advantage of speed and was used in the majority of experiments. Length measurements were used where appreciable growth inhibitions were expected.

(f) Culture media: Preliminary experiments were carried out to determine which of the media, sucrose, glucose and water is suitable for use in the experiments. Growth of sections in water continues only for the first ten hours, but in glucose and sucrose up to 48 to 52 hours. The growth in sucrose is better than that in glucose. So sucrose was selected as the compound for use as the medium. In order to determine the concentration of sucrose suitable for optimal growth a range of concentrations of sucrose was tested. It was found that  $\frac{1}{2}\%$  sucrose shows the best results. At this concentration of sucrose there will be little chance of bacterial contamination, so that reasonably sterile conditions will be maintained. (Audus and Garrard (1953).) The growth of sections in  $\frac{1}{2}\%$  sucrose solution is very uniform. The basic growth medium was  $\frac{1}{2}\%$  sucrose solution. The importance of appropriate supply of sugar in the growth of maize root sections was emphasised by Brown and Sutcliffe (1950). They say that sugar effect induces absorption of water, accumulation of sugar inside the tissue, synthesis of cellulose and enhancement of respiration.

The use of all buffering salts was avoided so that growth should not be affected by any inorganic ions. That buffer solutions produce considerable influences on the response of root tissue to auxin was shown by Thimann and Scheneider (1938), Brown and Sutcliffe (1950).

The volume of the culture solution was always 10 c.c. Under the optimal conditions of water supply, a total increase of about 220-240 per cent growth takes place in 48 hours.

(g) Growth condition: The presence of oxygen in the medium is most essential for maximum growth of sections. The roots are very sensitive to the oxygen tension in the medium for, even the slightest deficiency of it causes a marked reduction in growth. Coult (1939) demonstrated that agitation could markedly stimulate the growth of *Sinapis alba* roots. It had already been referred to that Audus and Garrard (1953) also obtained a statistically significant stimulation of the root section growth by allowing them to rock on an agitator.

(h) Selection of auxin concentrations

The 'physiological' auxin concentrations had been used during the course of experimentation, so that there could be no toxic effect due to high auxin concentrations. The concentrations selected for most experiments are one part in  $10^{11}$ , one part in  $10^{10}$  (both are stimulatory concentrations) and also one part in  $10^8$  which causes non toxic growth inhibition of the order of about 30%. The optimum concentration for stimulation is one part in  $10^{11}$ .

As a precautionary measure, against bacterial contamination, which profoundly depresses growth, the glass ware and



instruments were kept under absolute alcohol between experiments and were sterilised in hot air oven for 3 hours before use. The alcohol was changed from time to time.

## CHAPTER III

INVESTIGATION OF THE CAUSES OF VARIATION IN  
THE DEGREE OF STIMULATION BY IAA UNDER  
EXPERIMENTAL CONDITIONS

- (1) (a) The relationships between the age of the root and the sensitivity of sections to IAA.

It had been observed in previous experiments carried out by many workers in this laboratory (Bedford College) that there was a marked variation in response of root sections to the stimulatory concentrations of IAA. But in these lines no attempt was made to investigate the effect of age of root on this stimulation of IAA. The sensitivity of roots to added IAA might be markedly changed with age. It was, therefore, considered necessary to investigate this age effect on the response of root sections to IAA.

Plan of the experiment: A large number of sterile sand pots were taken. They were labelled with the dates. The soaked pea seeds were planted in these sterile sand pots. Pots were then transferred to constant temperature room and kept inside the dark cupboard. All seedlings were thus germinated in complete darkness at 25°C. in a saturated atmosphere. After 24 hours, a few pots were removed from the constant temperature room and sections were excised from extending zone of the radicles of one day old seedlings. The excised sections were treated with one part in 10<sup>11</sup>

and  $10^8$  IAA solution. Control samples in  $\frac{1}{2}\%$  sucrose solution were also observed at a time. The same procedure was adopted for studying the effect of the sensitivity of 2, 3, 4, 5 day old roots to IAA treatment. Three such experiments with two replications were conducted. The plan of the full investigation is given below. Growth of sections was measured after a period of 48 hours.

Table I  
Age effect

Plan of full investigation.

Total number of samples of 10 sections.

Conc.(gm/ml) IAA.	Age of the seedlings (in days) after planting				
0	1	2	3	4	5
	6	6	6	6	6
$10^{-11}$	6	6	6	6	6
$10^{-8}$	6	6	6	6	6

The ratios of the growths of treated samples to those of corresponding controls were calculated. The graphs shown (in Fig. 4) were drawn from the ratios of the growths against days for each concentration of IAA.

Figure 4

Graphs showing the effect of age of parent root on the response of excised sections to B-indolylacetic acid (1AA). The vertical line represents the least significant difference at the 5% probability level between any two points. The figure against the line records the number of replicate samples from which each mean was calculated.



GROWTH AS % OF CONTROL IN SUCROSE ALONE

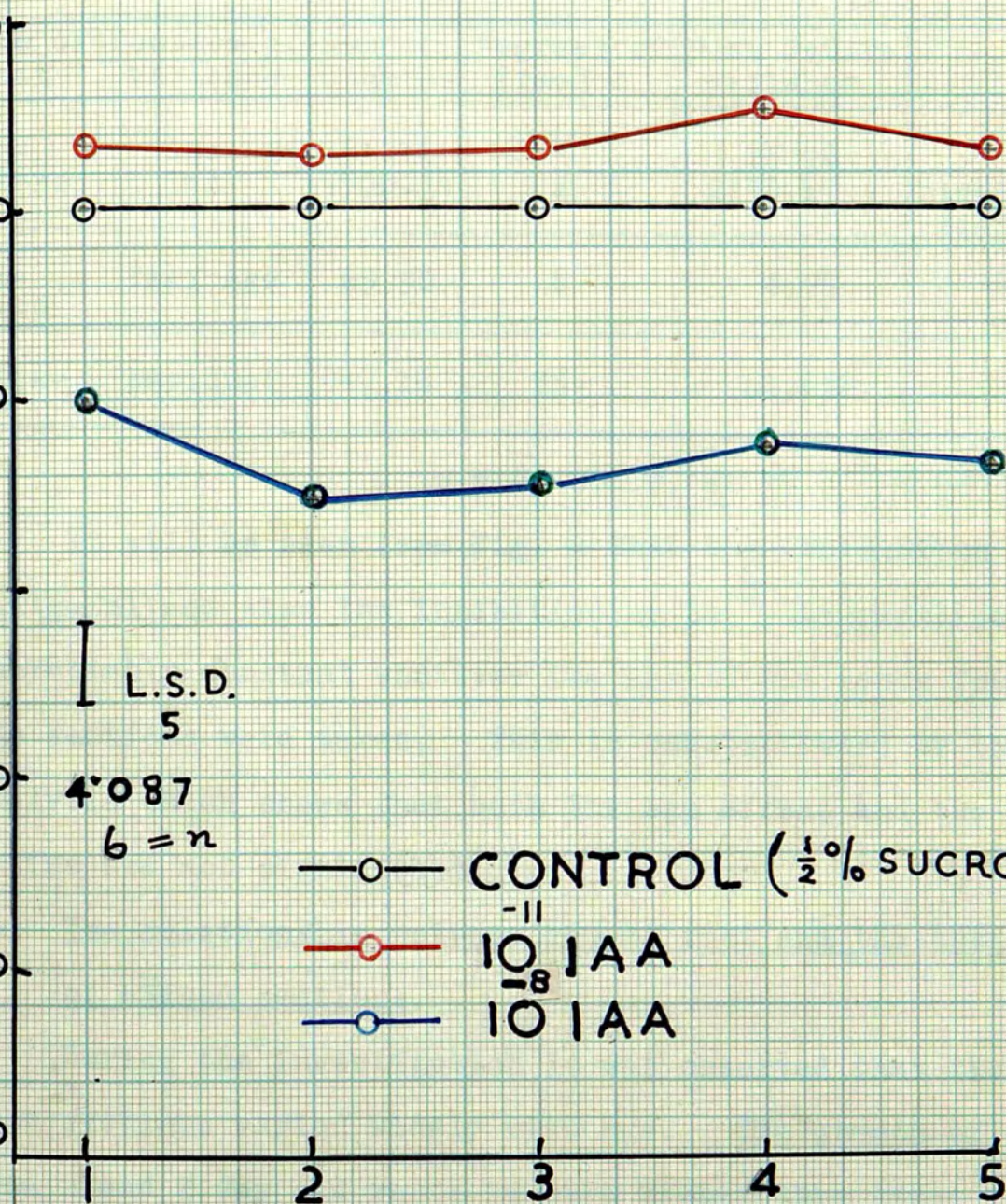
110  
100  
90  
80  
70  
60  
50

L.S.D.  
5  
4.087  
6 = n

—○— CONTROL ( $\frac{1}{2}\%$  SUCROSE)  
—○—  $10^{-11}$  IAA  
—○—  $10^{-8}$  IAA

TIME IN DAYS AFTER PLANTING

FIG 4





The results were subjected to analysis of variance and it is shown below.

Table 2.

The effect of age and IAA interation (Ratios)

Nature of effect	Sources of variance	Sum of Squares	Degrees of freedom	Mean Square variance	P.
Main factors	Age IAA	61.11 4700.34	4 2	15.28 2350.17	- highly significant
Inter-action	IAAX Age	69.98	8	8.75	
Replication	Residual	947.16	75	12.628	

$$n = 6, \text{ L.S.D. at } 5\% = \pm 4.087$$

It will be seen from Fig. 4 that the stimulation by  $10^{-11}$  IAA (gm/ml) and also the inhibition by  $10^{-8}$  IAA (gm/ml) seems to be appreciably constant whatever the age of the roots from which the sections were taken. This is substantiated by the variance analysis which shows that both the age effect and the inter-action are not significant.

(b) The relationship between age of root and the total growth of excised sections.

During the investigation it was observed that the growth of root sections varied widely with age. The growth of sections from 2 and 3 day old roots is much higher than those from 4 and 5 day old roots. Experiments were then repeated with two stimulatory concentrations of IAA (i.e. one part

in  $10^{11}$  and  $10^{10}$ ) and one inhibitory concentration which is (one part in  $10^8$ ). The growth (fresh weight) of sections was measured with micro-torsion balance. This time the total growth of the treated and control samples for 48 hour periods, were recorded and an analysis of variance of the results was performed. The graphs were drawn from the average of the total growth and they are shown in (Fig. 5).

Figure 5.

Graphs showing the effect of age(total growth) of parent root on the response of excised Sections to B-indolylacetic acid (1AA). The vertical line represents the least significant difference at the 5% probability level between any two means. The figure against this line records the number of replicate samples from which each mean was calculated.



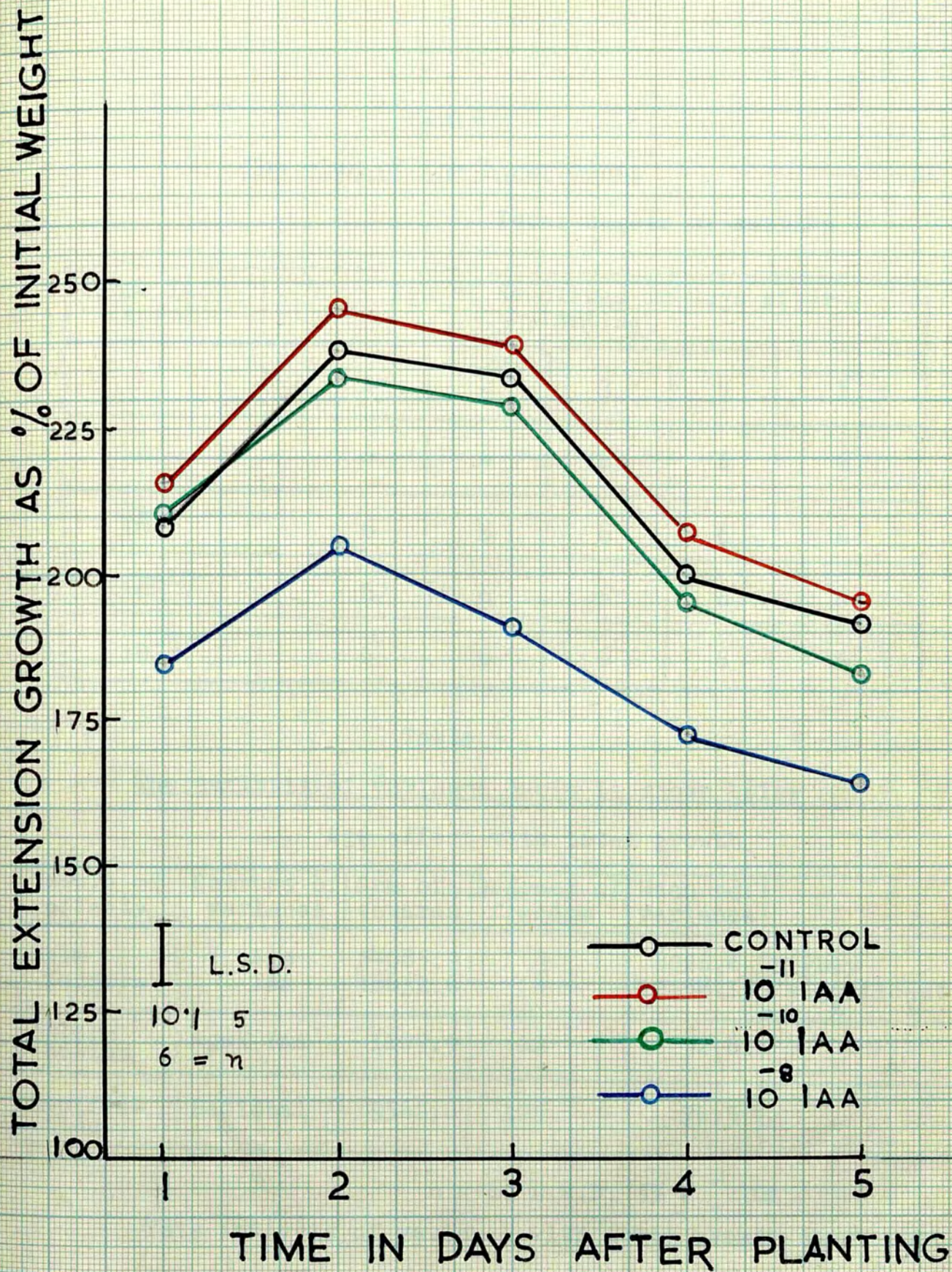


FIG 5



Table 3

The plan of the experiments

Total number of samples

conc. IAA gm/ml	Age of seedlings in days after planting				
	1	2	3	4	5
0	6	6	6	6	6
$10^{-11}$	6	6	6	6	6
$10^{-10}$	6	6	6	6	6
$10^{-8}$	6	6	6	6	6

Table 4

The analysis of variance table is shown below

Age effect with total growth

Nature of effects	Sources of variance	Sum of squares	Degrees of freedom	Mean sq. variance	P.
Main factors	Age IAA	24085.9 48632.6	3 4	8028.6 12158.1	highly significant " " "
Inter-action	AgeX IAA	1107.9	12	92.6	-
Replication	Residual	9562.0	125	76.5	

$$n = 6, \text{L.S.D. } 5\% = \pm 10.1$$

This analysis of variance with total growth shows that the effect of age is highly significant. The growth of

sections with age varies widely. The growth <sup>rate</sup> is highest in 2 and 3 day old roots but lowest in 4 and 5 day old roots (See Fig. 5). There is a marked decrease in rate of growth of sections in 4 and 5 day old roots. The reason for this sudden decline in growth rate of sections is unknown, it might be due to some changes in the metabolic products of the cells. The interaction between age and IAA is not significant. This shows that in spite of a pronounced effect of age, its influence on IAA response is nil.

Conclusion: Therefore it is suggested that the variations in response of sections to stimulatory concentrations of IAA observed in previous experiments by other workers in this laboratory is not due to variations in the age of the root materials used.

(2) (a) Studies on the effect of light on the stimulatory effects of IAA in the growth of root sections:

The earlier workers in this laboratory used to excise the root sections in diffuse sunlight. They observed a great variation in the stimulation by stimulatory concentrations of IAA. But nobody took notice of the fact that diffuse light might cause this marked variation on the stimulation of IAA. That light has a pronounced effect on the added IAA was already reported by Galston (1949, 1950). He found (1949) that etiolated stem sections

treated with IAA cultured in light always showed a marked depression of growth rate, but when such sections treated with IAA were grown in dark there revealed a pronounced stimulation. He, therefore, concluded that in light destruction of externally added IAA took place. In the presence of riboflavin this photo-inactivation was very much accelerated. Addition of  $Mn^{++}$  and  $cu^{++}$  ions to riboflavin stops this photo-inactivation of IAA.

Taking all these facts into consideration it was considered worth while to study the effect of light on the stimulatory concentrations of IAA in the growth of root sections.

Plan of the experiment: As a strict precautionary measure against any effect of light, diffuse or strong, the sand pots were well covered with black papers and seedlings were allowed to germinate in complete darkness inside the dark cupboard.

One series of samples to be grown in light were cut under electric light of intensity 100 watt. while the other series to be cultured in dark were cut in dim red light, since red light apparently has no effect on the inactivation of IAA.

After excision, the sections were treated with concentrations of IAA ranging from one part in  $10^{11}$  to one part in  $10^7$ . The series of samples which were grown in dark were covered with black papers. The other series were



put under fluorescent tubes. The intensity of light was measured with a photometer and it was 80 foot candle. The sections were thus allowed to grow in light and dark for a period of 48 hours. Three such experiments were performed. At the end of 48 hours the samples which were grown in light and dark were weighed separately in electric light and red light respectively.

The plan of the experiment is shown below.

Table 5

Effect of light on response of IAA

Plan of experiments

Total number of Samples.

conc. (gm/ml) IAA	Light	Dark
0	6	6
$10^{-11}$	6	6
$10^{-10}$	6	6
$10^{-9}$	6	6
$10^{-8}$	6	6
$10^{-7}$	6	6

The growths of treated samples in dark and light were calculated as ratios to the light control. The average of the samples were calculated and graphs were drawn against IAA concentrations for both light and dark. Results are reproduced in Fig. 6.

Figure 6

Graphs showing the light-effect on the response to B-indolylacetic acid (1AA) of the growth of excised root sections. The vertical line shows the least significant difference at the 5% probability level between any two means of ratios to light control. The figure against this line records the number of replicate samples from which each mean of the series was calculated.



GROWTH AS % OF CONTROL IN LIGHT

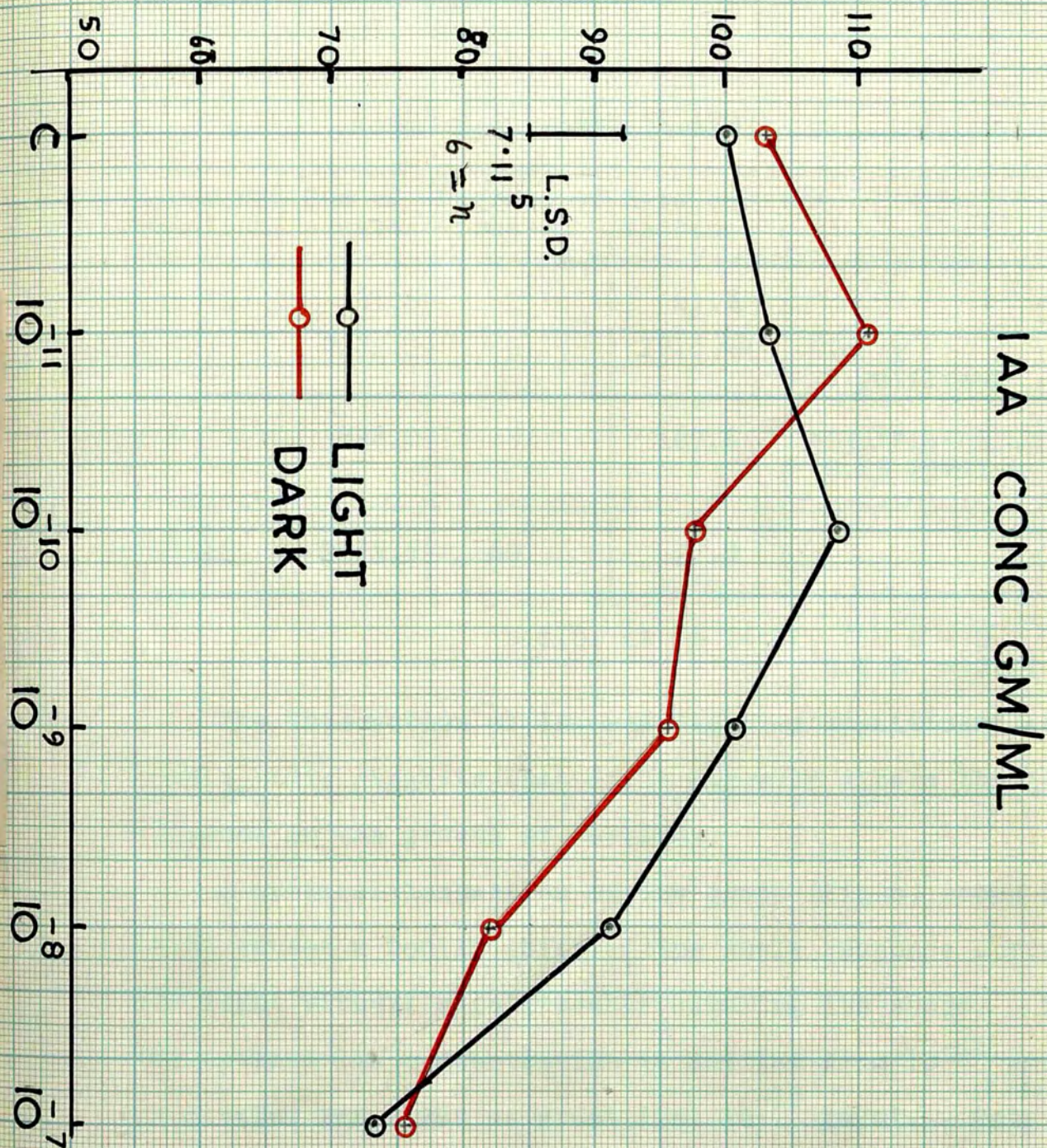


FIG 6



The results of the analysis of variance are shown below:

Table 6  
Light - IAA interaction

Sources of variation	Sum of squares	Degrees of freedom	Mean sq. variance	P
Light (L)	61.8	1	61.8	-
IAA (I)	9082.2	5	1816.44	highly significant
IXL	838.0	5	167.6	significant at 0.1% level
Residual	2225.5	60	37.9	

L.S.D. at 5% level, for  $n = 6$ ,  
L.S.D. = 7.11

The interaction of IAA and light is significant at 0.1% level; this suggests that light markedly alters the IAA response in the growth of sections.

A critical inspection of the graph (Fig.6) shows that light causes a bodily shift of the whole response curve to the right without significantly altering its shape. Thus the optimum IAA concentration is shifted from  $10^{-11}$  gm/ml to a value ten times as great i.e.  $10^{-10}$  gm/ml. Only at  $10^{-7}$  gm/ml IAA is there no effect of light on response.

The degree of stimulation is not altered by light but apparent effectiveness of the IAA is greatly reduced possibly



due to photo-inactivation.

The variation in the stimulation observed in earlier experiments could not have been due to an effect of diffuse light, since in those experiments the optimum effect was seen at  $10^{-11}$  gm/ml IAA <sup>corresponding to</sup> the dark optimum in my experiments.

(b) Studies on the effect of light on IAA response in presence of Manganese

The photo-inactivation of IAA as reported by Galston (1949) has already been discussed. The addition of very little quantity of ribo flavin caused rapid inactivation of IAA in light. This IAA-inactivation is blocked when  $Mn^{++}$  or  $Cu^{++}$  ions were added to this riboflavin. He envisaged that flavo protein enzyme produced  $H_2O_2$  which was utilised by IAA-peroxidase. When  $Mn^{++}$  ion was added to this process, it decomposed  $H_2O_2$  produced by flavo protein before peroxidase could use this  $H_2O_2$  to oxidise IAA. Thus the destruction of IAA was prevented.

It was found in my above experiments that light causes a shift of the optimum from one part in  $10^{11}$  to one part in  $10^{10}$  and it alters the sensitivity of root to added IAA. These results raise one important question. Is this altered sensitivity of root to auxin in light a real sensitivity change or are the results merely due to auxin destruction? If light inactivates auxin and auxin is present in sub-optimal concentrations (since added auxin stimulates growth) then

light should depress growth of roots. Therefore light effect may likely to be an effect on sensitivity of root to auxin. To check the above hypothesis the effect of light and IAA was studied in presence of manganese ions.

Exactly the same experimental procedure was adopted. Since in my last experiment there was no marked effect of light, in this experiment, the intensity of light was increased to 120 foot candle, which might be quite strong enough to produce some effect on the growth rate of the sections. The sections were cut in red and electric light.

Two concentrations, i.e. one part in  $10^{11}$  gm/ml and one part in  $10^{10}$  gm/ml of IAA, were used alone and in combination with ( $10^{-5}$  molar) solution of manganese. The treated samples were grown in light of intensity 120 foot candle and also in the dark.

The plan of the experiment is shown below.

Table 7

IAA- light interaction in presence of  $Mn^{++}$  ion

Plan of the experiment

Total number of samples

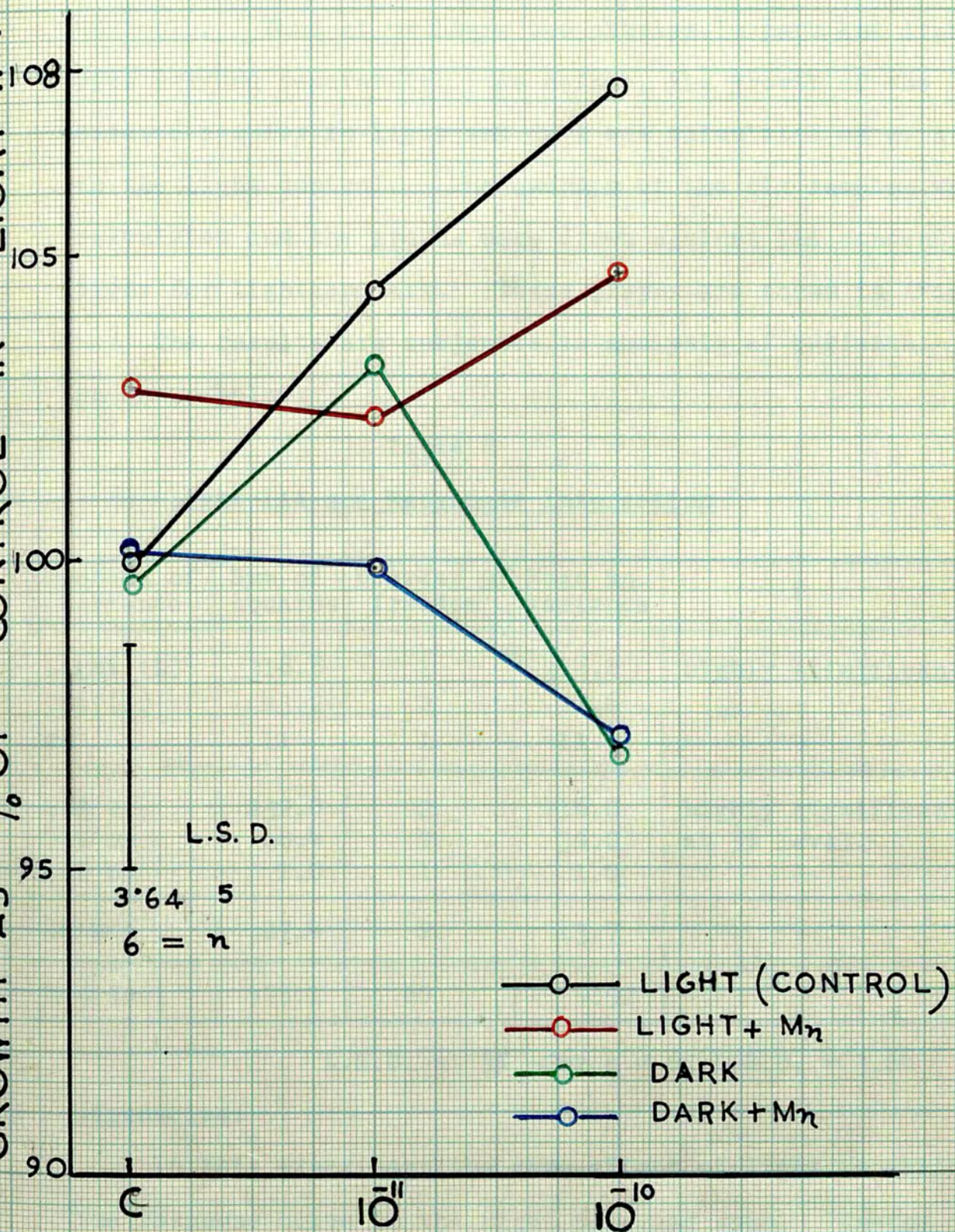
Conc. (gm/ml) IAA	Light (120 foot candle)		Dark	
	0	$10^{-5}$ M.M <sub>n</sub>	0	$10^{-5}$ M.M <sub>n</sub>
0	6	6	6	6
$10^{-11}$	6	6	6	6
$10^{-10}$	6	6	6	6

Figure 7

Graphs showing the effects of 1AA in presence of  $10^{-5}$  M. manganese on the growth of the excised root sections in light and dark. The vertical line shows the least significant difference at the 5% probability level between any two means of the ratios to Light Controls. The figure against this line records the number of replicate samples from which each mean was calculated.



GROWTH AS % OF CONTROL IN LIGHT WITHOUT  $M_n$



IAA CONC GM./ML.

FIG 7



The growth of sections were followed for a period of 48 hours. Then at 48 hours the samples in dark and light were weighed separately. As before, the growth of the treated samples in light and dark was calculated as ratios to the light control. The average of the six samples was calculated and the graphs were drawn against IAA concentrations for light and dark. These graphs are shown in (Fig.7).

The results are subjected to analysis of variance. It is shown below:

Table 8

Light-IAA and Manganese Interaction

Sources of variance	Sum of squares	Degrees of freedom	Mean sq. variance	P
Light (L)	318.8	1	318.8	highly significant
IAA (I)	43.2	2	21.6	-
Mn	11	1	11	-
L X I	232.0	2	116.0	highly significant. at 5% level.
I X Mn	60.4	2	30.2	
L X Mn	1.0	1	1.0	
LXIXMn	27.4	2	13.7	-
Residual	595.1	60	9.92	

L.S.D. at 5% for  $n = 6$ , L.S.D. = 3.64

Discussion:

In this experiment the same effect of light in shifting the response curve to the right is again seen. In the dark

in the absence of Mn a stimulation is seen at  $10^{-11}$  gm/ml IAA, which <sup>has</sup> just reached significance level (cf. with L.S.D.5. in Fig.7) whereas in the light a much larger stimulation peak is seen at  $10^{-10}$  gm/ml IAA. This is clearly substantiated by the highly significant light X IAA interaction (Table 6). In the presence of Mn the graph suggests that there is no significant effect of IAA either in the light or in the dark and this is substantiated by the significant IAA X Mn interaction and supported by the high (although not quite significant) light X IAA X Mn triple interaction. With the exception of the rather high value for the control manganese in the light, these results suggest that manganese opposes the stimulation of root growth by IAA independantly of the lighting conditions and this does not support the theory of the photoinactivation of IAA put forward by Galston. These results however are insufficient for a well-founded disproof of the theory and should be further amplified.

It is possible therefore that the whole change in sensitivity to auxin in light may be a real change in the protoplasmic sensitivity and not due to IAA destruction.

(c) Pre-illumination of root sections for first four hours followed by IAA treatment and grown in dark

This experiment was performed in order to check further the previous hypothesis, i.e. if there is a real change in sensitivity then pre-illuminated sections grown in dark should show it. If it is due to auxin destruction then pre-illumination should not show it.

Of the two series of samples, one series was put under electric light of intensity 120 foot candle, and the other lot was allowed to grow in dark inside dark cupboard. As before, sections were cut in red light and strong electric light of intensity 100 watt.

The growth of the sections were followed for first four hours. At the end of (0-4) hours they were then removed and weighed. The rate of growth (fresh weight) per hour was calculated. The graph was drawn from these rates. It is shown in (Fig. 8A).

An analysis of variance was performed to test the effect of light treated for first four hours. This is shown below:

Table 9

Pre-illumination for first four hours  
No IAA

Sources of variance	Sum of Squares	Degrees of Freedom	Mean sq. variance	P.
Light (L)	119.4	1	119.4	highly significant
Expt. (E)	3.4	2	1.7	—
EXL	0.2	2	0.1	—
Residual	45.2	66	0.68	

$$n = 36. \quad \text{L.S.D.} = .388$$

The effect of light is highly significant. The growth rate of sections in light is much higher than in dark. The both series of samples were subsequently treated with one part in  $10^{11}$  and  $10^{10}$  of IAA. The light was put off and all the samples were grown in dark. The growth of sections



Figure 8

Graph A shows the effect of illumination of root sections for 4 hours.

Graphs B, C and D show the response to applied B-indolylacetic acid (1AA) subsequent to illumination. The vertical lines represent the least significant difference at the 5% probability level between any two means. The figure against these lines records the number of replicate samples from which each mean was calculated.

D = Dark.

Dp = pretreatment in dark.

L = Light.

Lp = pretreatment with light.



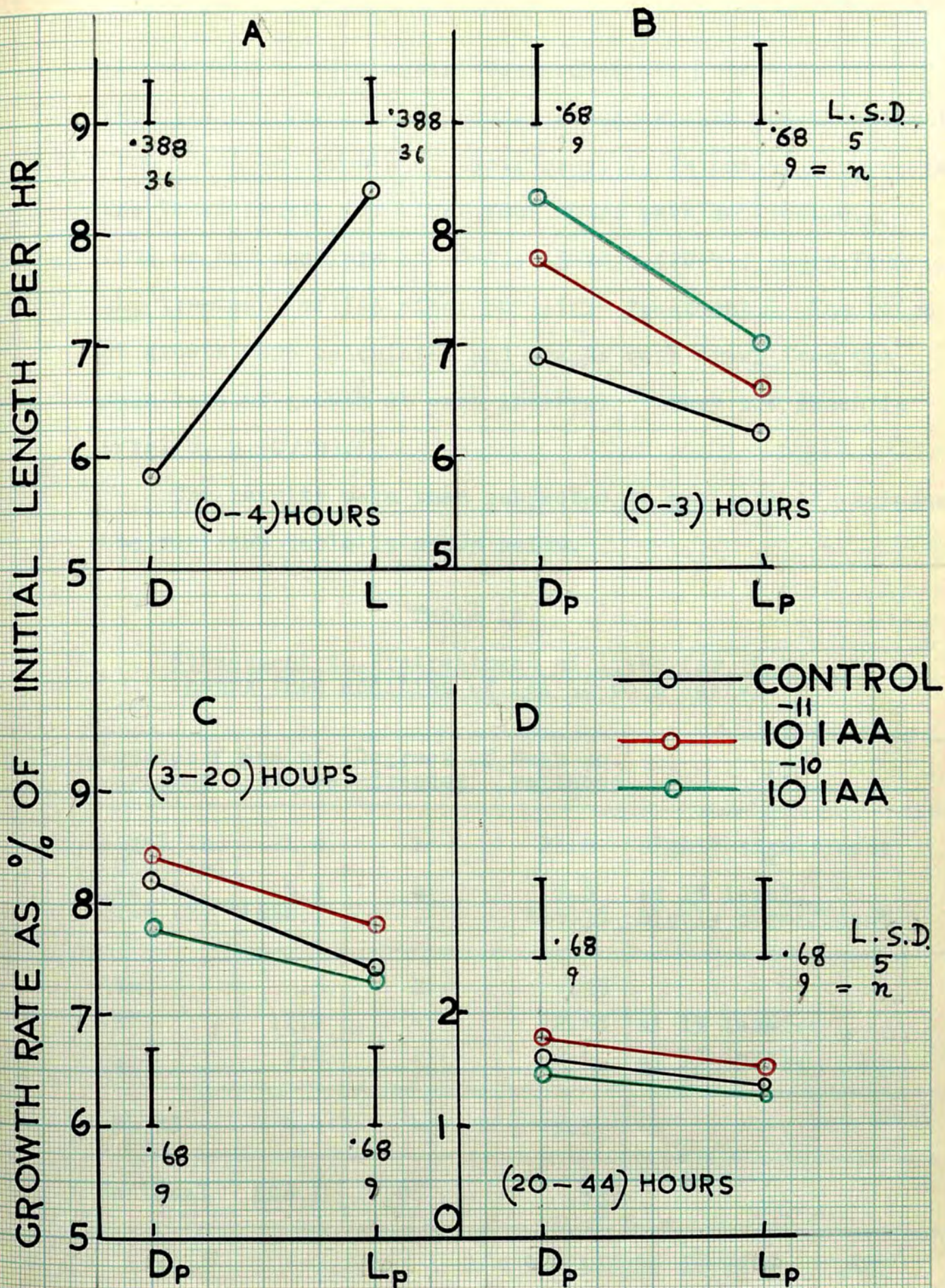


FIG 8



(fresh weight) were again observed at the intervals of (4-7), (7-24) and (24-48) hours, subsequent to pre-illumination. From the total growth of sections at 7, 24 and 48 hour period the total growth for first four hours were subtracted. Thus we had readings at (0-3), (3-20), (20-44) hours from the treatment of IAA after the light treatment. The rates of growth were calculated and graphs were drawn from the mean rate of growth at each interval. They are shown in (Fig. 8, B, C, D).

The results were then subjected to analysis of variance; the variance table is shown below.

Table 10

Subsequent to Pretreatment of light for (0-4)  
hours then addition of IAA to both series

Sources of variance	Sum of squares	Degrees of freedom	Mean sq. variance	P.
Light (L) (Pretreated)	21.6	1	21.6	highly significant.
IAA (I)	5.2	2	2.6	at 1% level.
Time (T)	1680.7	2	840.35	highly significant.
IXL	.5	2	.25	-
IXT	11.4	4	2.85	at 1% level.
LpXT	5.2	2	2.6	at 1% level.
IXL <sub>p</sub> XT	4.4	4	1.1	-
Residual	106.1	198	.535	

n = 9    L.S.D. at 5% = .687



Fig. 8 B, C and D and the variance analysis show that light pretreatment causes a significantly lower growth rate of sections in the dark. The cause of this is obscure but it seems probable that it is due to a residual effect of illumination on the growth system itself. The most important result, however, is that the IAA effects are independent of pre-illumination (IAA X light interaction insignificant). In addition it will be seen that  $\frac{1}{10^{-10}}$  gm/ml IAA there is a marked stimulation in the first 3 hours after pre-illumination and this becomes a slight inhibition over the 3-20 hour period. This time shift of response is also independent of pretreatment illumination conditions. We must therefore abandon the theory that the altered IAA sensitivity in light is a direct action in the protoplasm and revert to the IAA photoinactivation theory. But the action of Mn in this photoinactivation would not seem to be that postulated by Galston.

(3) Metallic contamination and its influence on the stimulatory effect of IAA in the growth of root sections

Introduction:

It was Robbins (1922) who first gave a demonstration of the effects of various nutrient solutions on the growth of excised root tips. He showed that the excised root in suitable nutrient solutions grow as luxuriously and as

rapidly as it would have had it been attached to the intact plants. White (1934) found that transference of excised root sections to various sub-cultures improved the growth of the sections. Using a medium adequate in vitamins, he (1943) demonstrated that a deficiency of iron from the nutrient medium caused complete cessation of growth of root sections. Omission of manganese, boron, zinc and iodine also led to reduction of growth rate but not complete cessation. He suggested that some concentration of these elements is essential for optimal growth of the tomato root. Addition of  $\text{ZnCl}_2$ ,  $\text{MnCl}_2$  and borax of 0.1 p.p.m. is beneficial for the growth of root sections. (Robbins *et al.* 1936).

The trace elements used in White's medium (1943<sup>4</sup>) are:  
 (in mg/liter)  $\text{KI}$ , 0.75;  $\text{Fe}_2(\text{SO}_4)_3$ , 2.5,  $\text{MnSO}_4$ , 4.5,  
 $\text{ZnSO}_4$ , 1.5; and  $\text{H}_3\text{BO}_3$ , 1.5.

Eltinge and Reed (1940) found that absence of zinc from the medium caused to develop some abnormal symptoms in the growth of excised tomato roots. Glasstone's (1947) intensive work on the effects of micro-elements on the growth of excised root brought to light the fact that iron and copper in dilute concentrations are essential for the normal growth of roots.

Boll and Street (1951) found poor growth of excised tomato roots in White's medium prepared at Manchester, when

similar medium prepared at Nottingham supported good growth. This observation prompted them to investigate for the real cause of this variation in the growth of <sup>root</sup> sections in two batches, but prepared at different places. It was later on discovered by them that this variation in growth of root was due to deficiency of micro nutrients present in different batches of double-distilled water, and of the A.R. grade Salts used. They found that addition of small amounts of copper as copper sulphate, and of molybdenum as molybdic acid to the medium resulted in improvement in root growth. A range of concentration of copper at (0.01, 0.02, and 0.04 p.p.m.) exerts a stimulatory effect on both main axis length and number of laterals per root. The effect of molybdenum alone also had a significant stimulatory effect. In presence of 0.01 p.p.m. added copper, molybdenum at all concentrations (0.005 to 0.05) <sup>P.P.M.</sup> gave significant growth increases in all the features measured.

This observation gives a clear indication that the growth-promoting activity of White's medium varies with nature and amount of heavy metal contamination. They also suggested that in addition to this unknown metallic nutritive factors required for the growth of excised roots and such factors can occur to a variable extent as contaminant in inorganic salts.

Miller (1952,1954) obtained a marked stimulatory effect of cobaltous chloride on the growth of excised stem sections.



Cobaltous chloride in presence of sugar and IAA produced stimulation of the order of 30% over the untreated control. (That potassium nitrate causes stimulation in growth of maize roots was shown by Brown and Sutcliffe (1950).) It was, therefore, considered necessary to study the effect of the metallic contaminations that may arise in ordinary distilled water on the stimulatory effects of IAA in the growth of root sections.

The heavy metals that <sup>are</sup> likely to occur in ordinary distilled or tap water :-  $\text{Mn}^{++}$ ,  $\text{Ca}^{++}$ ,  $\text{Pb}^{++}$ ,  $\text{Zn}^{++}$ ,  $\text{Cu}^{++}$ ,  $\text{Fe}^{++}$ ,  $\text{Mg}^{++}$  and boron etc.

In studies of the effect of heavy metal impurities, tri-glass distilled water free from all metallic contaminations, was used in the medium as well as in making dilutions. The detailed account of the preparation tri-glass distilled water is given in introduction, (p.p. 18)

#### (I) (a) Effect of Calcium alone:

Action curve: The various range of concentrations of  $\text{CaCl}_2$  were made. The range of concentrations selected for use in studying the action curve was from one part in  $10^8$  to one part in  $10^4$  molar solutions. 2-m.m. long excised sections of root were allowed to grow in this range of concentrations of  $\text{CaCl}_2$  for a period of 48 hours. Three experiments with two replications were performed.

The plan of the experiment is shown below:

Table 11

Plan of Experiments

Expt.	Concentrations of Calcium chloride (molar)					
	0	$10^{-8}$	$10^{-7}$	$10^{-6}$	$10^{-5}$	$10^{-4}$
E1	2	2	2	2	2	2
E2	2	2	2	2	2	2
E3	2	2	2	2	2	2

At the end of 48 hours, the sections were weighed and the percentage increase in Fresh weight was noted. The ratios of the growths of the treated samples to those of corresponding controls were calculated. The action curve shown in (Fig. 9A) was drawn from the average of these ratios against range of concentrations of  $\text{CaCl}_2$  used.

(b) The effects of Calcium in combination with IAA

The above range of concentrations from ( $10^{-8}$  to  $10^{-4}$  M) of  $\text{CaCl}_2$  were used in combination with two concentrations of IAA, i.e. one part in  $10^{11}$  and one part in  $10^8$ , the former is a stimulatory concentration and latter is an inhibitory one.

Figure 9A

Graph showing the action curve of Calcium on the growth of root sections over 48 hours. The vertical line represents the least significant difference at the 5% probability level between any two means. The figure against this line records the number of replicate samples from which each mean of the series was calculated.



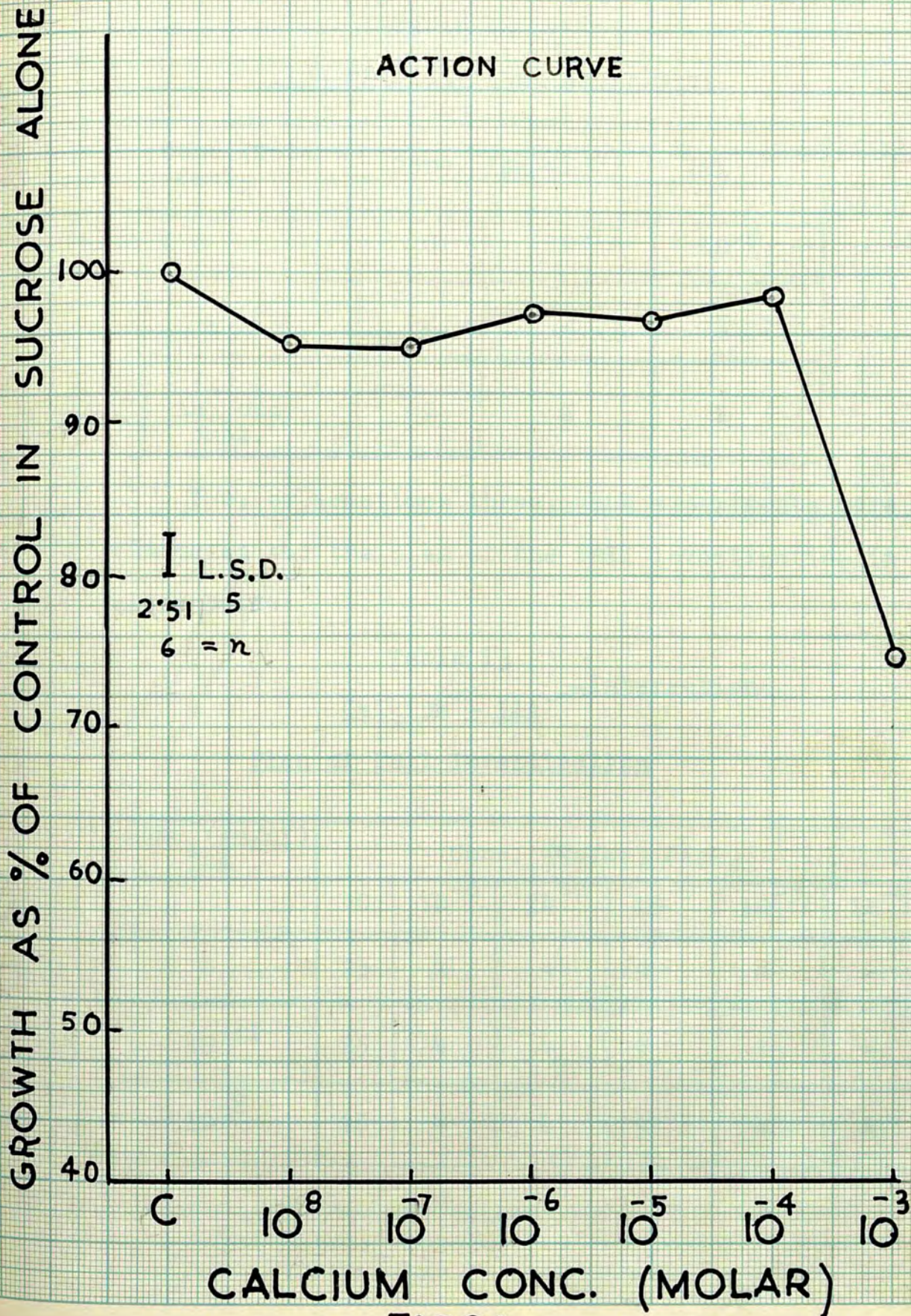


FIG 9A



The plan of the individual experiment is shown below:

Table 12

Plan of Individual Experiment

Conc. (Molar) CaCl <sub>2</sub>	Conc. (gm/ml) IAA		
	0	10 <sup>-11</sup>	10 <sup>-8</sup>
0	2	2	2
(x) any one conc.	2	2	2

Two, or sometimes three, such experiments were conducted and the plan of the full investigation is given below:

Table 13

Calcium - IAA <sup>Interaction</sup>

Plan of Full Investigation

conc. (gm/ml) IAA	Conc. (Molar) CaCl <sub>2</sub>					
	0	10 <sup>-8</sup>	10 <sup>-7</sup>	10 <sup>-6</sup>	10 <sup>-5</sup>	10 <sup>-4</sup>
0	25	4	4	9	4	4
10 <sup>-11</sup>	25	4	4	9	4	4
10 <sup>-8</sup>	25	4	4	9	4	4

Figure 9B

Graphs showing the effect of Calcium on the growth response to B-indolylacetic acid (1AA) of root sections over 48 hours. The vertical lines represent the least significant differences at the 5% probability level between the means of the ratios to corresponding controls at the particular concentrations of Calcium against which they are placed. The figures against these lines record the number of replicate samples from which each mean of the series was calculated.



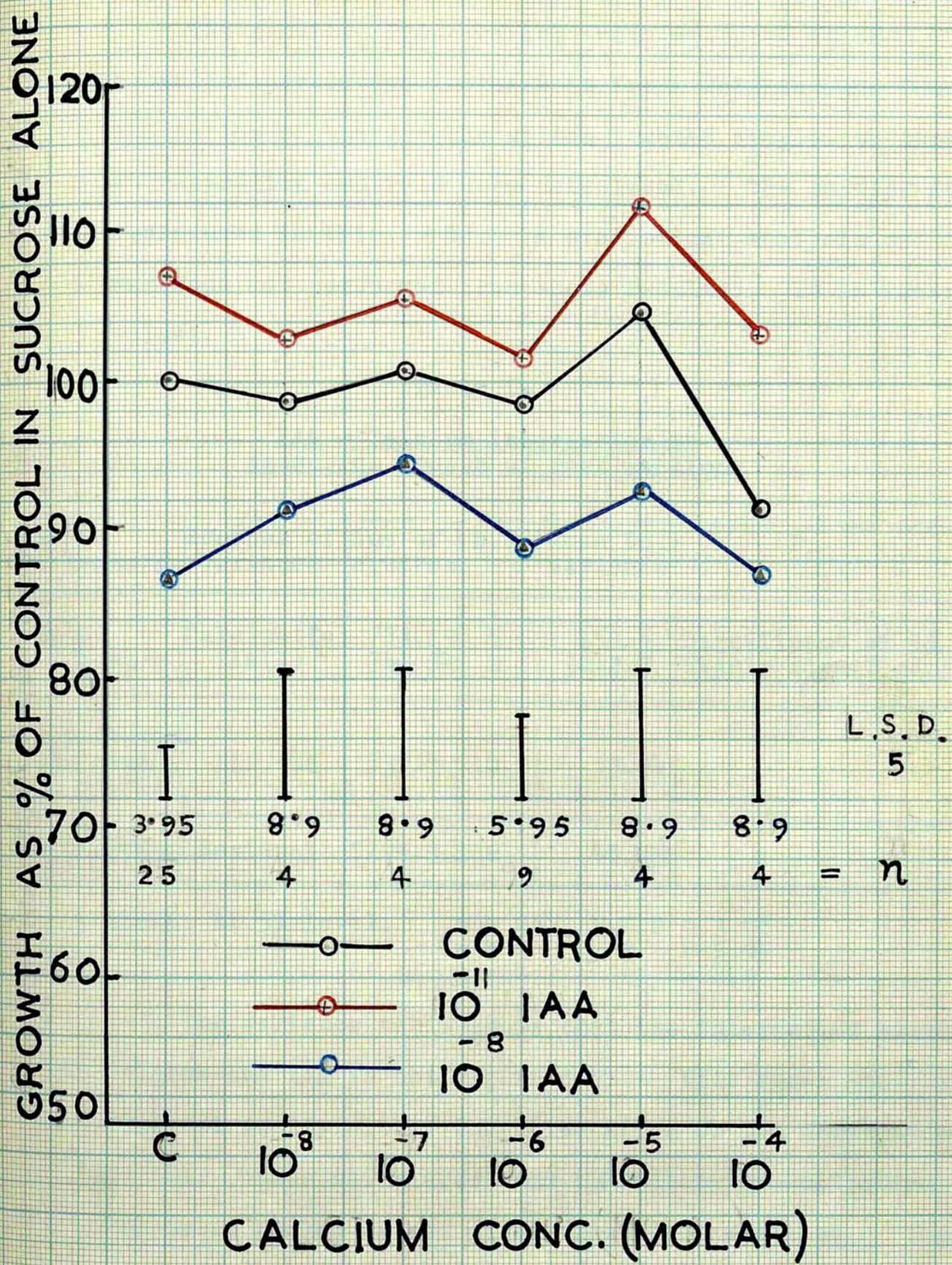


FIG 9B



The growths (Fresh-weight) were measured at 48 hours and percentage increase in fresh weights were found. The ratios of the growths of the treated samples to those of corresponding controls were calculated. The results are reproduced in (Fig. 9 B). A statistical analysis of variance was performed from those results. It is shown below:

Table 14

IAA - Calcium interaction in  
extension growth of root sections

Main factors	Sources of variance	Sum of squares	D.F.	Mean Sq. variance	P.
Main factors	Calcium(Ca) IAA(I)	753.29 762.54	5 2	150.6 3812.7	Significant at 1% level. Highly significant
Interaction between pairs	IX Ca	535.4	10	53.54	
Replication	Residual	5294.3	132	40.109	

L.S.D. at 5% level.      n = 25      L.S.D = 3.95  
                                  n = 9          "      = 5.95  
                                  n = 4          "      = 8.9

The variance table shows that the interaction between Calcium and IAA is not significant. This shows that Calcium does not modify either the stimulation or the inhibition due to IAA. Both stimulatory and inhibitory concentrations of IAA cause marked effect on the growth of sections. Therefore, the effect of IAA is seen to be highly significant. The

overall effect of Calcium is significant and can be shown to be due to a small stimulation at  $10^{-5}$  M and a small inhibition at  $10^{-4}$  molar.

#### Discussion:

These results do not agree well with those reported by Burström (1952 and 1954). He showed very marked stimulatory effect of calcium on the growth of wheat root. He said that calcium is involved in the metabolism of cell wall, such, as in the formation of pectic materials of the middle lamella. Calcium, in his experiment, is shown to act as though an antiauxin. No such effect is observed in our experiments. This insignificant effect of calcium may be explained that in pea root tissue almost optimum amounts of calcium are already in the root for which further addition of calcium causes no marked growth increase.

#### Conclusion:

The results of experiments lead us to conclude that calcium impurities in the culture solution does not alter auxin response.

#### (II) (a) The effect of manganese alone

As it was done in the previous experiment, a range of concentration from ( $10^{-8}$  -  $10^3$  M) solution of manganese chloride was used. The root sections were grown in these solutions, in complete darkness, at temperature  $25^{\circ}\text{C}$ , and in saturated humidity inside the incubator. The plan of the



experiments is given below. The growth of the sections was followed for a period of 48 hours:

Table 15  
Action curve of Manganese  
 Plan of the experiment

Expt.	Conc. (molar) manganese						
	0	$10^{-8}$	$10^{-7}$	$10^{-6}$	$10^{-5}$	$10^{-4}$	$10^{-3}$
I	2	2	2	2	2	2	2
II	2	2	2	2	2	2	2

Increase in growth (fresh weight) of sections after a period of 48 hours was measured and from this data the percentage increase was calculated. The ratios of the growth of treated samples to those of corresponding controls were worked out. This result is reproduced in Fig. 10 A (Action curve of Manganese).

When the action curve of manganese was determined, the investigation on the effect of manganese to the response of IAA was carried out as follows:-

(b) The effect of manganese on the response of IAA

Two concentrations (one part in  $10^{11}$  and one part in  $10^8$ ) IAA were used in combination with concentrations of manganese chloride ranging from ( $10^{-8}$ – $10^{-4}$ M), solution.

Figure 10A

Graph showing the action curve of Manganese on the growth of root sections over 48 hours. The vertical line represents the least significant difference at the 5% probability level between any two means. The figure against this line records the number of replicate samples from which each mean of the series was calculated.



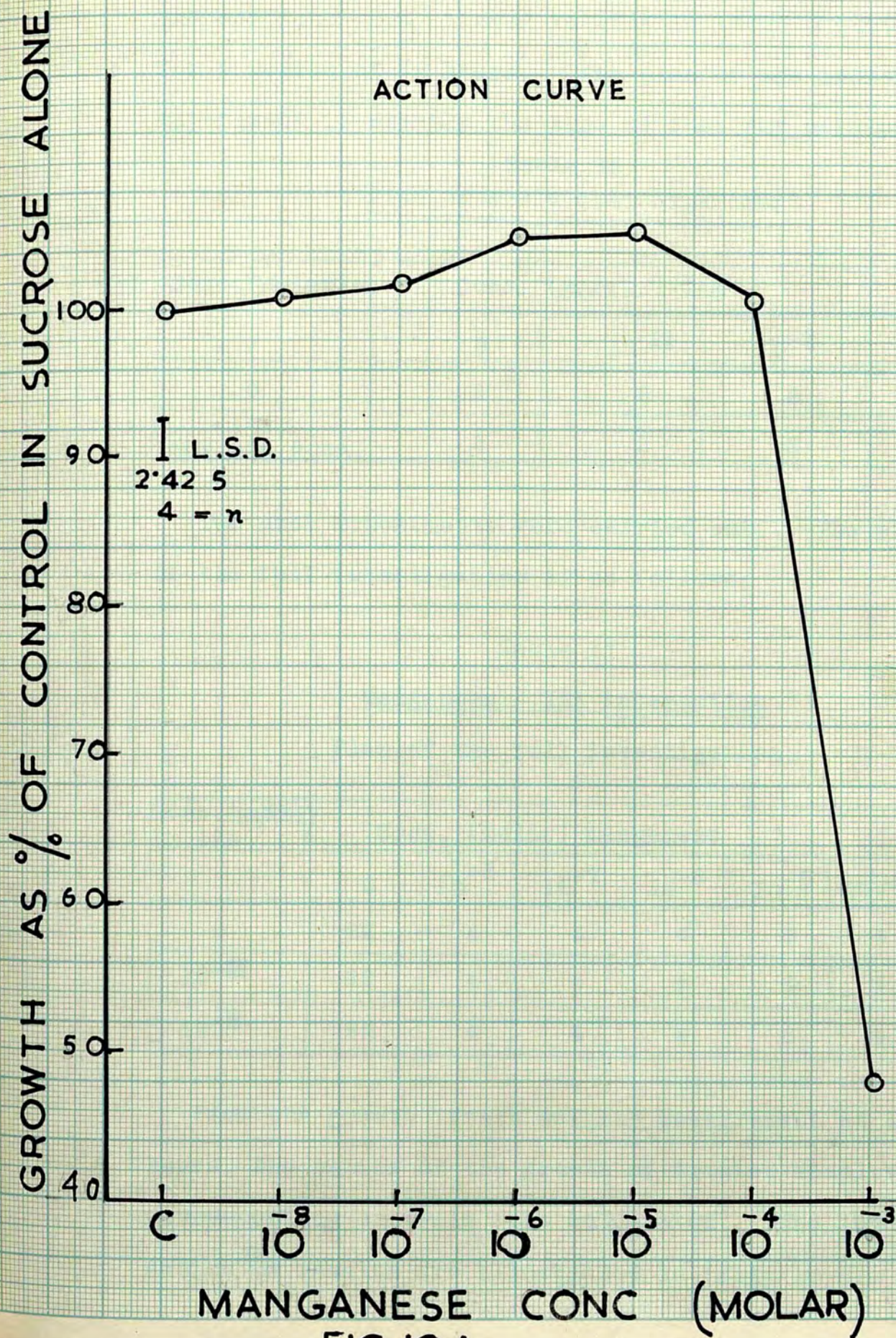


FIG 10A



The sections were allowed to grow in these solutions for a period of 48 hours. The plan of the individual experiment is exactly the same as that for calcium.

Table 16  
Plan of individual experiment

Conc. (Molar) $M_n$	Conc. (gm/ml.) IAA		
	0	$10^{-11}$	$10^{-8}$
0	2	2	2
(x) any one conc.	2	2	2

Two such experiments were carried out. The plan of the full investigation is as follows:

Table 17  
Manganese - IAA interaction  
Plan of the full investigation.

Conc. (gm/ml.) IAA	Conc. (molar) manganese					
	0	$10^{-8}$	$10^{-7}$	$10^{-6}$	$10^{-5}$	$10^{-4}$
0	22	4	4	4	6	4
$10^{-11}$	22	4	4	4	6	4
$10^{-8}$	22	4	4	4	6	4

From the growth (fresh weight) measurement at 48 hours, the ratios of the growth of treated samples to corresponding controls were calculated. The interaction <sup>of</sup> results are illustrated in (Fig. 10 B).

Figure 10B

Graphs showing the effect of Manganese on the growth response to B-indolylacetic acid (1AA) of root sections over 48 hours. The vertical lines represent the least significant differences at the 5% probability level between the means of the ratios to corresponding controls at the particular concentrations of Manganese against which they are placed. The figures against these lines record the numbers of replicate samples from which each mean of the series was calculated.



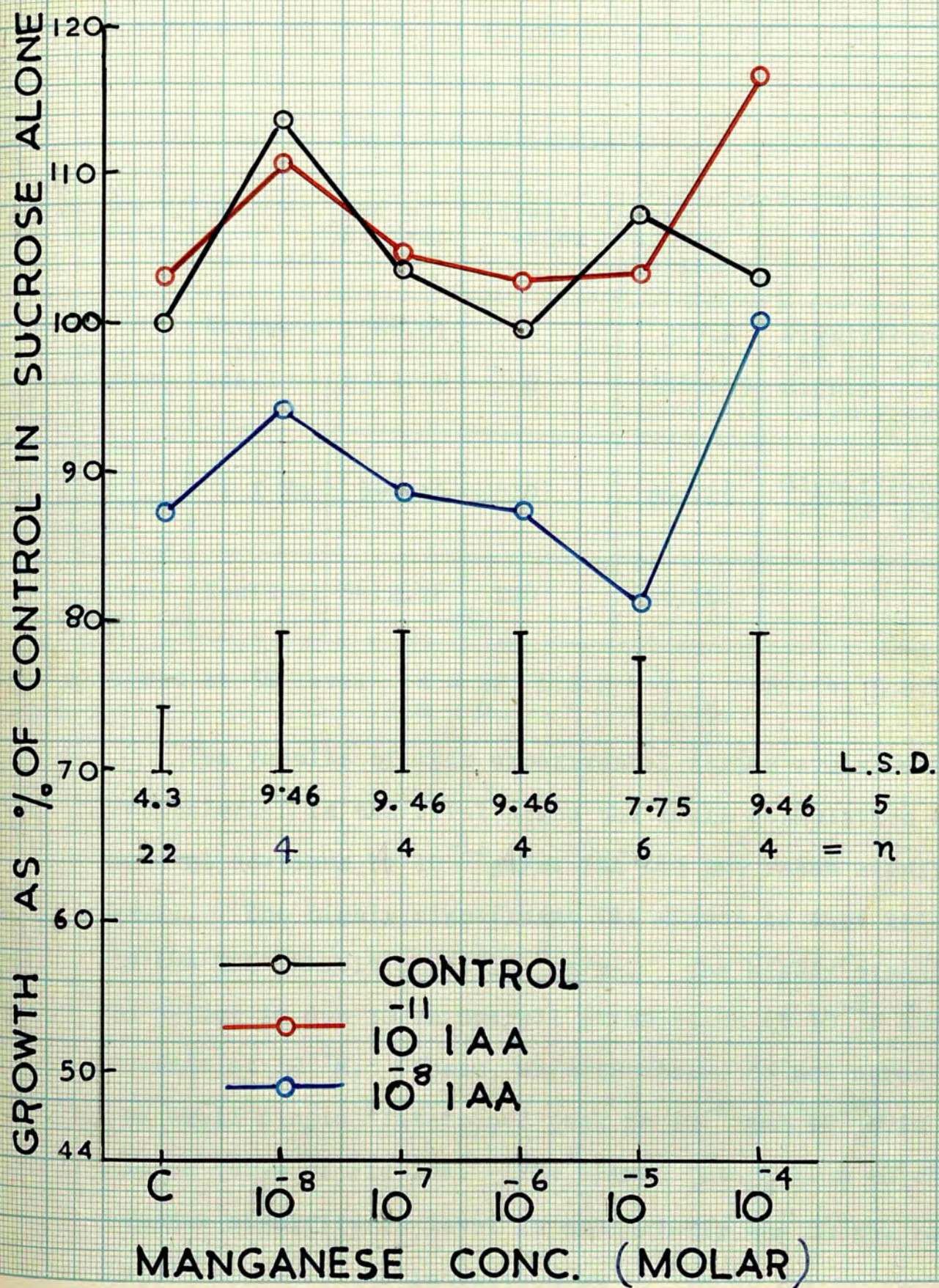


FIG 10B



Necessary analysis of variance of the results was performed and the table is shown below:

Table 18

Mn - IAA Interaction

Nature of effect	Sources of variance	Sum of Squares	Degrees of freedom	Mean Square Variance	P.
Main Factors	Manganese (Mn)	1588.01	5	317.6	Significant at 1% level
	IAA (I)	6313.19	2	3156.59	Highly significant
Inter-action between pairs	I X Mn	897.15	10	89.715	Significant at 5% level
Replication	Residual	5412.28	120	45.10	

L.S.D at 5% level  $n = 22$  L.S.D = 4.3  $n = 4$ , L.S.D = 9.46  
 $n = 6$  " = 7.75

The results of this experimental series are somewhat confusing. In the first place the small stimulation of control sections by  $10^{-11}$  gm/ml IAA did not reach significance level. The highly significant IAA effect is thus solely due to the IAA inhibition at  $10^{-8}$  gm/ml. The highly significant Mn effect is due to an overall stimulation at  $10^{-8}$  M and another at  $10^{-4}$  M. These, unfortunately, do not agree with the small single flat-topped peak at  $10^{-6}$  and  $10^{-5}$  M in the action curve of Fig.10A. The IAAxMn interaction which just reaches significance level is undoubtedly due to the responses in  $10^{-4}$  Mn where the inhibition by  $10^{-8}$  gm/ml IAA is abolished

but normal stimulation by  $10^{-11}$  gm/ml IAA reappears (cf. results in Fig. 7). It seems probable therefore that this experiment was exceptional in every way and needs careful repetition and checking.

(III) (a) The effect of zinc alone (action curve)

A wide range of concentrations ( $10^{-10}$ - $10^{-4}$ M) of  $ZnCl_2$  was used in determining the effect of  $Zn^{++}$  on the growth of pea root sections. The plan of the experiment is given below:

Table 19  
Action Curve of Zinc

Expt.	Conc. (molar) Zinc							
	0	$10^{-10}$	$10^{-9}$	$10^{-8}$	$10^{-7}$	$10^{-6}$	$10^{-5}$	$10^{-4}$
I	2	2	2	2	2	2	2	2
II	2	2	2	2	2	2	2	2
III	2	2	2	2	2	2	2	2

At 48 hours, the sections were weighed. The action curve is drawn from the ratios of the growth of treated samples to corresponding controls. It is shown in (Fig. 11A). There is no stimulation by zinc at any concentration ( $10^{-10}$ - $10^{-5}$ M). At  $10^{-4}$ M zinc causes a marked inhibition of about 30%.

(b) The effect of zinc on response of IAA

For the study of interaction of IAA and zinc, concentrations ( $10^{-7}$ - $10^{-4}$ M) of zinc were used in



Figure 11A

Graph showing the action curve of Zinc on the growth of the root sections over 48 hours. The vertical line represents the least significant difference at the 5% probability level between any two means. The figure against this line records the number of replicate samples from which each mean of the series was calculated.



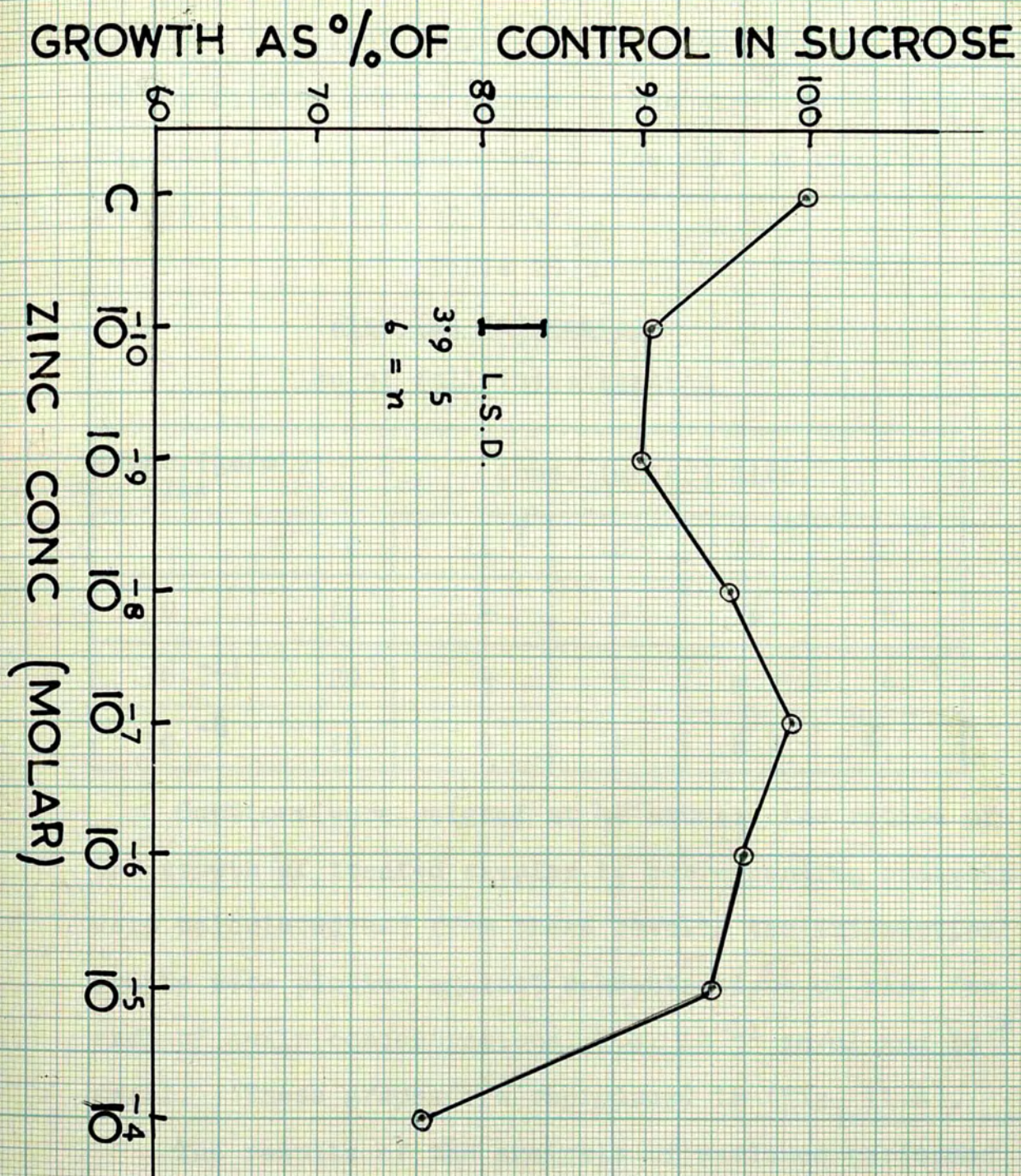


FIG 11A



combination with two concentrations of IAA ( $10^{-11}$  -  $10^{-8}$  gm/ml). The individual plan of the experiment is the same as for calcium and manganese. Two experiments with two replications were conducted. The plan of the full investigation is given below:

Table 20

Zinc-IAA Interaction

## Plan of full investigation

Conc. (gm/ml) IAA	Conc. (molar) Zinc				
	0	$10^{-7}$	$10^{-6}$	$10^{-5}$	$10^{-4}$
0	20	4	4	6	6
$10^{-11}$	20	4	4	6	6
$10^{-8}$	20	4	4	6	6

Sections were weighed at 48 hours: the ratios of the growth of treated samples to corresponding controls were calculated and from these results the interaction graphs were plotted against concentrations of zinc for each of auxin concentrations, i.e.  $10^{-11}$  gm/ml and  $10^{-8}$  gm/ml. These graphs are reproduced in (Fig. 11 B).

The results of the analysis of variance performed on the ratios were shown on the next page:

Figure 11B

Graphs showing the effect of Zinc on the growth ~~and~~ response to B-indolylacetic acid (1AA) of excised root sections over 48 hours. The vertical lines represent the least significant differences at the 5% probability level between the means of the ratios to corresponding controls at the particular concentrations of Zinc against which they are placed. The figures against these lines record the numbers of replicate samples from which each mean of the series was calculated.



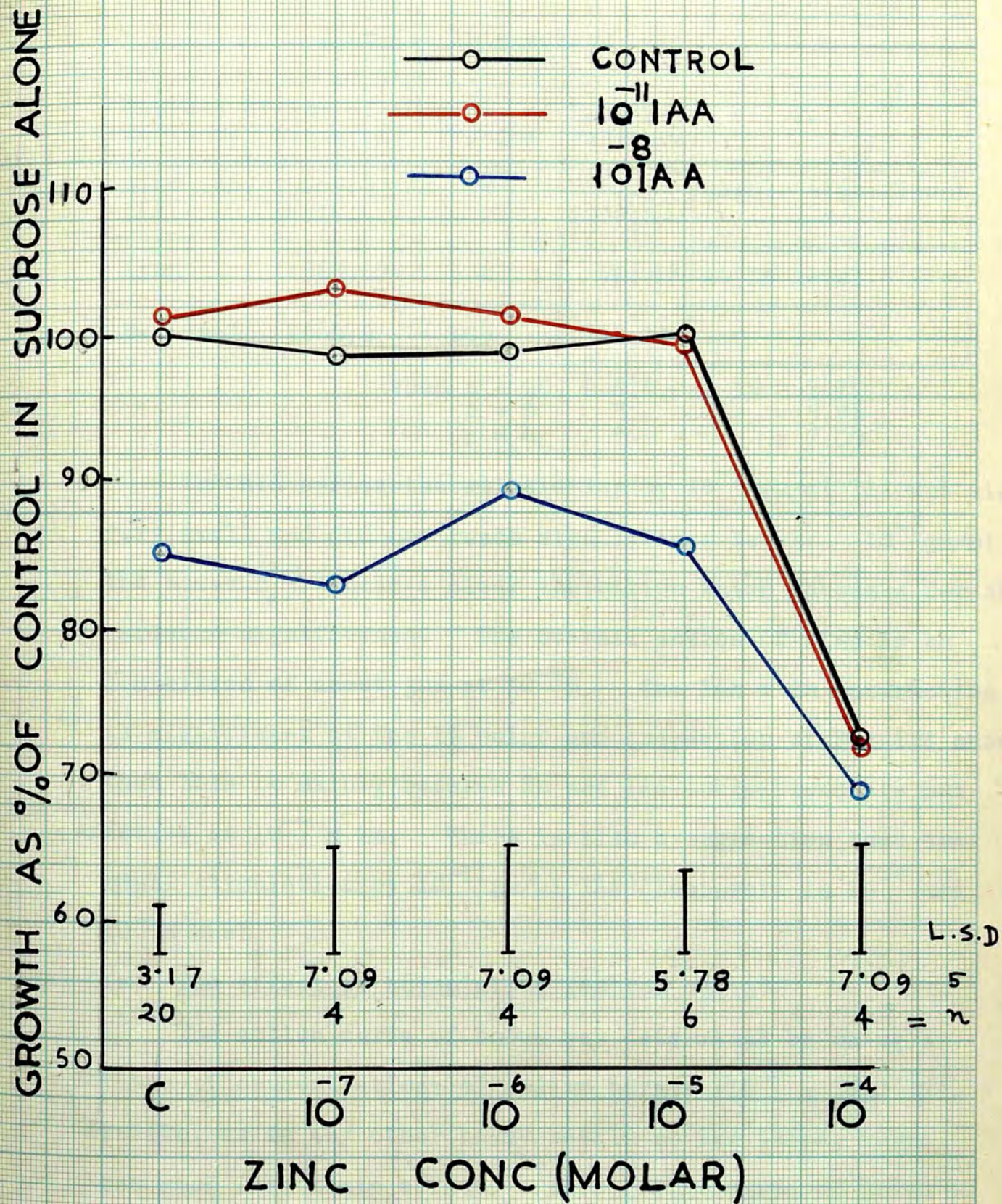


FIG II B



Table 21  
Zinc-IAA Interaction

Sources of variance	Sum of squares	Degrees of freedom	Mean Sq. variance	P.
Zinc (Zn)	9069.33	4	2267.3	highly significant
IAA	4514.4	2	2257.2	"
IxZn	565.67	8	70.718	at 1% level
Residual	2666.0	105	25.39	

when  $n = 20$ , L.S.D. at 5% = 3.17  
 $n = 6$  " = 5.78  
 $n = 4$  " = 7.09

In this series the stimulation by  $10^{-11}$  gm/ml IAA was also very small and did not reach significance level.  $10^{-8}$  gm/ml IAA however, produced a clear inhibition which accounts for the variance significance. The overall action of zinc is an inhibition of about 30% at  $10^{-4}$  M. The IAA x Zn interaction is significant at the 1% point and inspection of Fig. 11B shows that this is mainly because Zn and IAA inhibitions are not additive at  $10^{-4}$  M Zn. There is also a suggestion that the small stimulation by  $10^{-11}$  gm/ml IAA disappears in  $10^{-5}$  and  $10^{-4}$  M  $\text{ZnCl}_2$ .

(IV) (a) The effect of boron alone (action curve)

The action curve of boron was determined by using a range of concentrations of sodium borate ( $10^{-9}$  -  $5 \times 10^{-3}$  M). Unlike, as in any other heavy metals so far tried, boron has much less toxic effect. This action curve of boron is established by conducting two experiments with two



replications. The plan of experiments is shown below:

Table 22

Action Curve of Boron

Plan of the experiments

Experiment	Conc. (Molar) of Boron								
	0	$10^{-9}$	$10^{-8}$	$10^{-7}$	$10^{-6}$	$10^{-5}$	$10^{-4}$	$10^{-3}$	$5 \times 10^{-3}$
1	2	2	2	2	2	2	2	2	2
2	2	2	2	2	2	2	2	2	2

The growth (fresh weight) of the sections were weighed at 48 hours and percentage increase was calculated. The ratios of the growths of treated samples to their corresponding control samples were worked out. The action curve of boron is drawn from these ratios. The action curve is shown in (Fig. 12A).

From the action curve it is quite clear that even  $5 \times 10^{-3} \text{M}$ . solution of sodium borate is not very toxic whereas in calcium, manganese and zinc, even the concentration  $10^{-4}$  molar is very much inhibitory.

(b) The effect of boron in combination with IAA

Two concentrations one part in  $10^{11}$  and  $10^8$  of IAA were used in combination with a range of concentrations of sodium borate ( $10^{-6}$  -  $10^{-4} \text{M}$ ). The plan of

Figure 12A

Graph showing the action curve of Boron on the growth of excised root sections over 48 hours. The vertical line represents the least significant difference at the 5% probability level between any two means. The figure against this line records the number of replicate samples from which each mean of the series was calculated.



# GROWTH AS % OF CONTROL IN SUCROSE

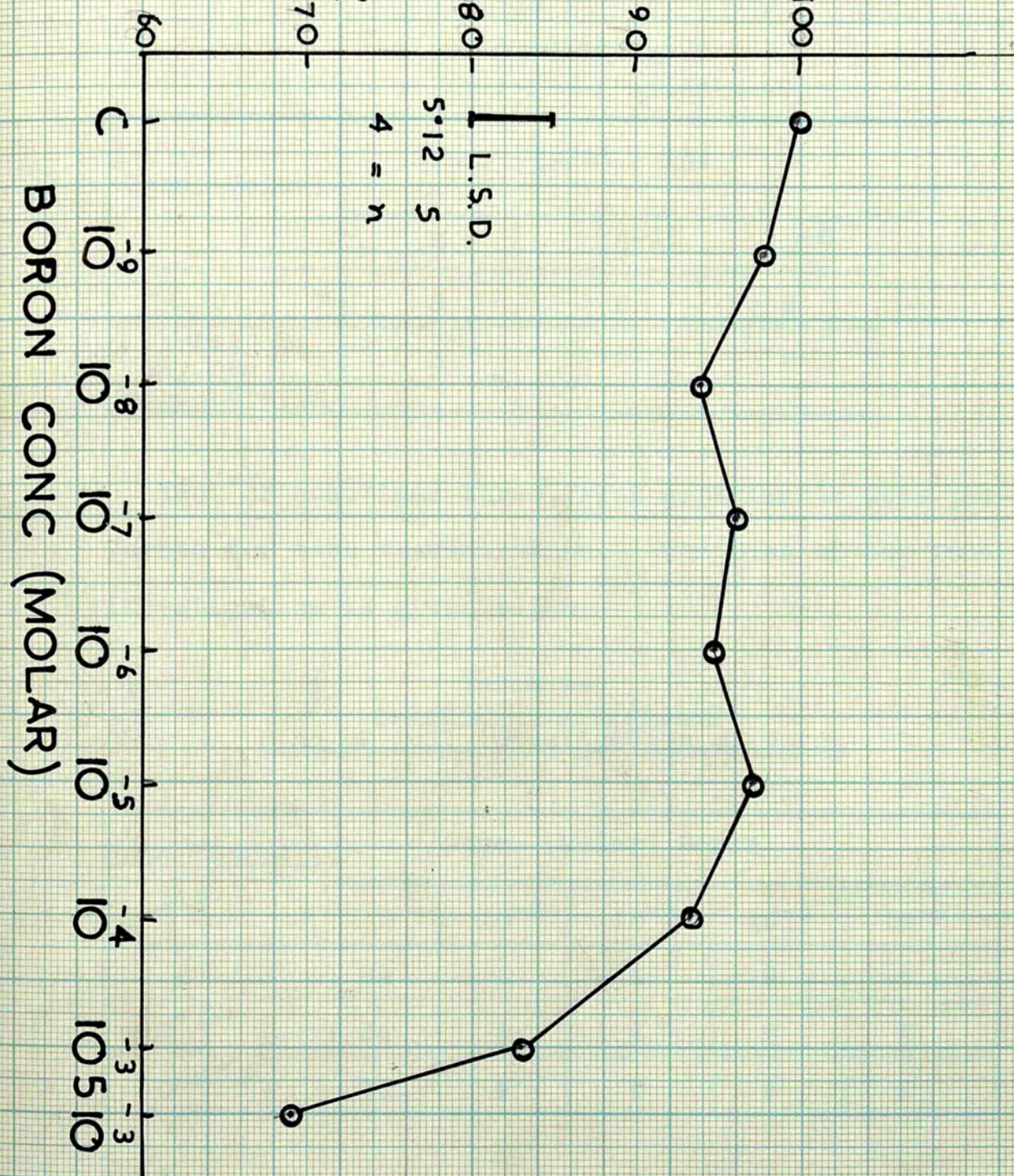


FIG 12 A



individual experiment is shown below:

Table 23  
IAA-Boron Interaction  
Plan of individual experiment

Conc. (Molar)	Conc. (gm/ml.) IAA		
	0	$10^{-11}$	$10^{-8}$
0	2	2	2
(x) any one concentration	2	2	2

Two, or sometimes three, such experiments were performed for each concentration. The plan of the full investigation is given below:

Table 24  
IAA-Boron Interaction  
Plan of full investigation of boron

Conc. (gm/ml.) IAA	Conc. (Molar) Boron				
	0	$10^{-6}$	$10^{-5}$	$10^{-4}$	$10^{-3}$
0	24	4	4	9	6
$10^{-11}$	24	4	4	9	6
$10^{-8}$	24	4	4	9	6

The ratios of the growths (fresh weight) of the treated samples to corresponding controls were calculated from the results observed at 48 hours. The graphs



Figure 12B

Graphs showing the effect of Boron on the growth and response to B-indolylacetic acid (1AA) of root sections over 48 hours. The vertical lines represent the least significant differences at the 5% probability level between the means of the ratios to corresponding controls at the particular concentrations of Boron against which they are placed. The figures against these lines record the numbers of replicate samples from which each mean of the series was calculated.



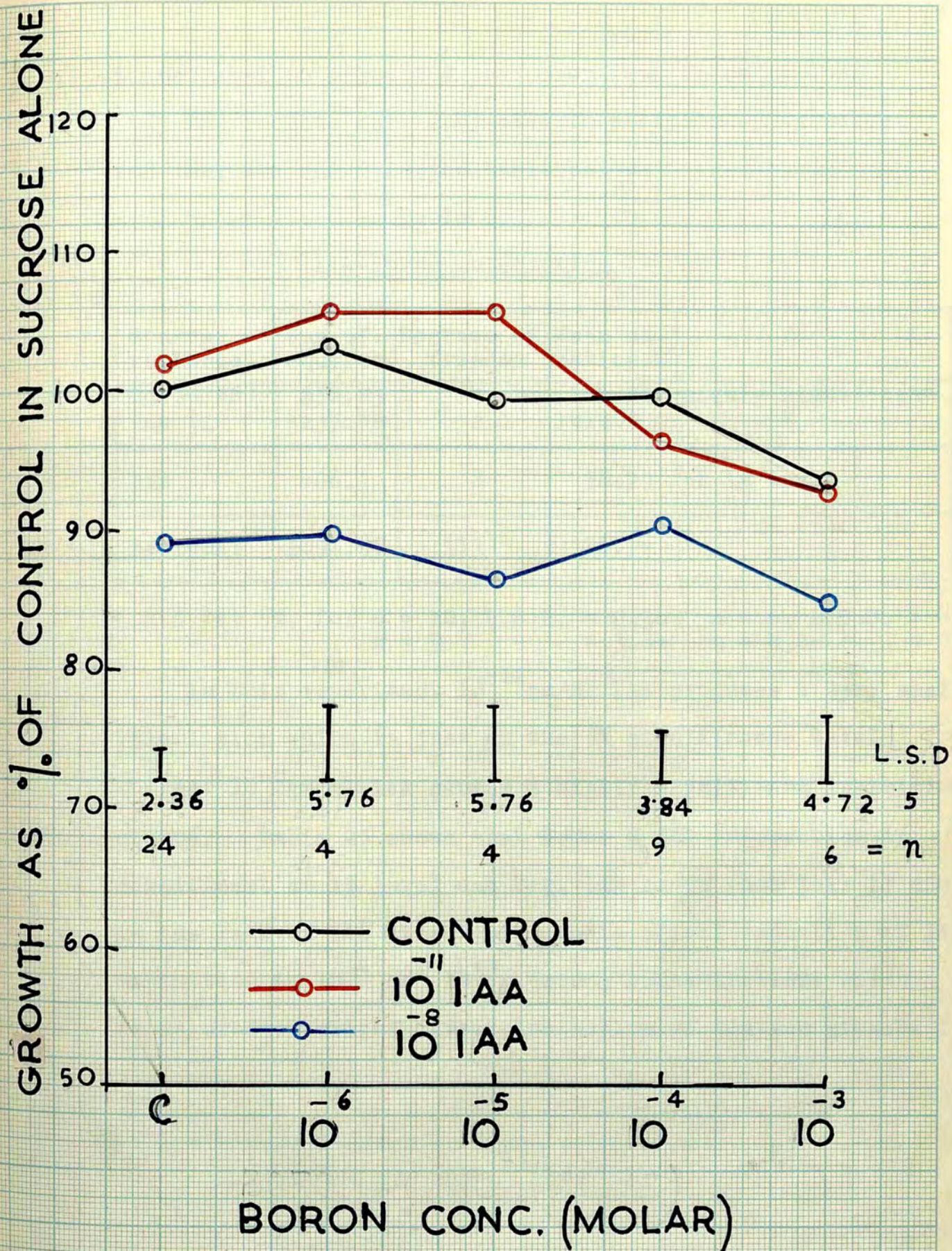


FIG 12 B





(V) Cobalt effectIntroduction:

Miller (1952, 1954) reported a very interesting effect of cobalt on the expansion of etiolated bean leaf and also on the promotion of elongation of etiolated pea sections. Cobalt in combination with sugar and IAA, causes much greater effect. Such a striking discovery prompted me to study the effect of cobalt on the growth of pea root sections itself and its influence on the stimulation of IAA.

(a) The effect of Cobalt alone (action curve):

As before it was thought necessary to try the effect of cobalt chloride solution alone on the growth of <sup>root</sup> sections. To this end a range of concentrations, from one part in ( $10^8$  to  $10^4$  M) of cobaltous chloride was used. The plan of experiments is given below:

Table 26  
Action curve of Cobalt  
Plan of Experiment

Expt.	Conc. (Molar) Cobalt chloride					
	0	$10^{-8}$	$10^{-7}$	$10^{-6}$	$10^{-5}$	$10^{-4}$
I	3	3	3	3	3	3
II	3	3	3	3	3	3



Figure 13A

Graph showing the action curve of Cobalt on the growth of excised root sections over 48 hours. The vertical line represents the least significant difference at 5% probability level between any two means. The figure against the line records the number of replicate samples from which each mean of the series was calculated.



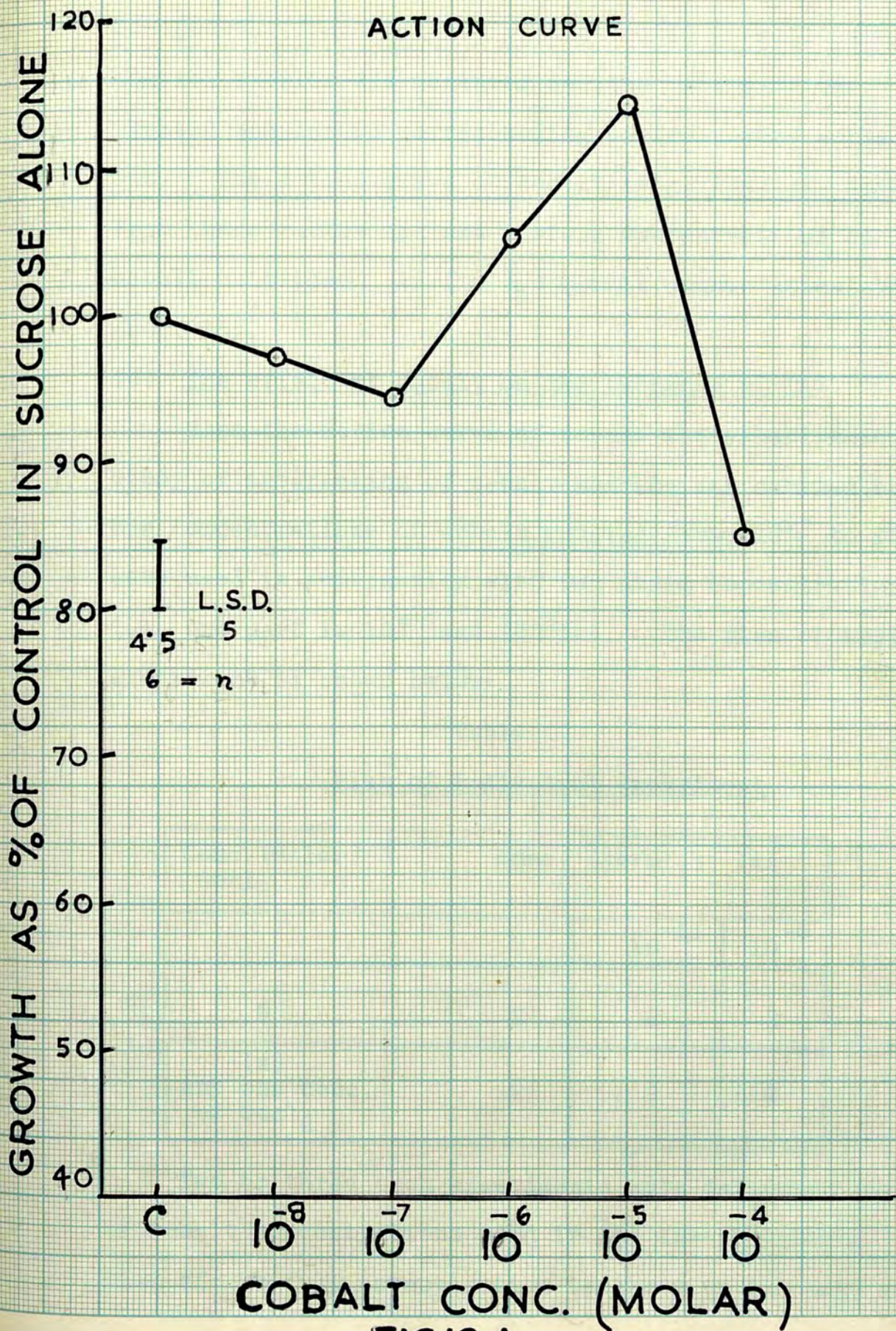


FIG 13 A



The growth (fresh weight) of the treated as well as control samples was measured and the ratios of the treated samples to corresponding controls were worked out. The action curve of cobalt (Fig. 13A) was drawn from these ratios against various concentrations of cobalt chloride. At ( $10^{-5}_M$ ) Cobalt causes stimulation of about 15%.

(b) The effect of Cobalt on the response of IAA

When the action curve of cobalt was established, the study of interaction of cobalt and IAA was carried out. The concentrations of cobalt ranging from ( $10^{-7}$  to  $10^{-4}_M$ ) were used in combination with two auxin concentrations, i.e. one part in  $10^{+11}$  and  $10^{-8}$  IAA. The plan of individual experiment is shown below:

Table 27

Plan of Individual Experiment

Conc. (Molar) Cocl <sub>2</sub>	Conc. (gm/ml.) IAA		
	0	$10^{-11}$	$10^{-8}$
0	2	2	2
(x) Any one conc. of Cocl <sub>2</sub>	2	2	2

Two or three such experiments were performed for each of cobalt concentrations used in the investigation. The plan of the full investigation is shown on the next page:

Table 28  
Cobalt-IAA Interaction  
 Plan of full investigation

Conc. (gm/ml.) IAA	Conc. (molar) cobaltous chloride				
	0	$10^{-7}$	$10^{-6}$	$10^{-5}$	$10^{-4}$
0	20	4	4	6	6
$10^{-11}$	20	4	4	6	6
$10^{-8}$	20	4	4	6	6

Following the above scheme the investigation on the interaction of cobalt and IAA was carried out and increase in fresh weight of the sections was measured at the end of 48 hours. The ratios of the growths of treated samples to corresponding controls were calculated. The interaction curves were drawn from these results against various concentrations of cobaltous chloride for each of IAA concentrations. They are illustrated in Fig. 13B.

The analysis of variance was performed from those results. This variance table is shown on the next page:



Figure 13B

Graphs showing the effect of Cobalt on the growth response to B-indolylacetic acid (1AA) of root sections over 48 hours. The vertical lines represent the least significant differences at the 5% probability level between the means of the ratios to corresponding controls at the particular concentrations of Cobalt against which they are placed. The figures against these lines record the numbers of replicate samples from which each mean of the series was calculated.



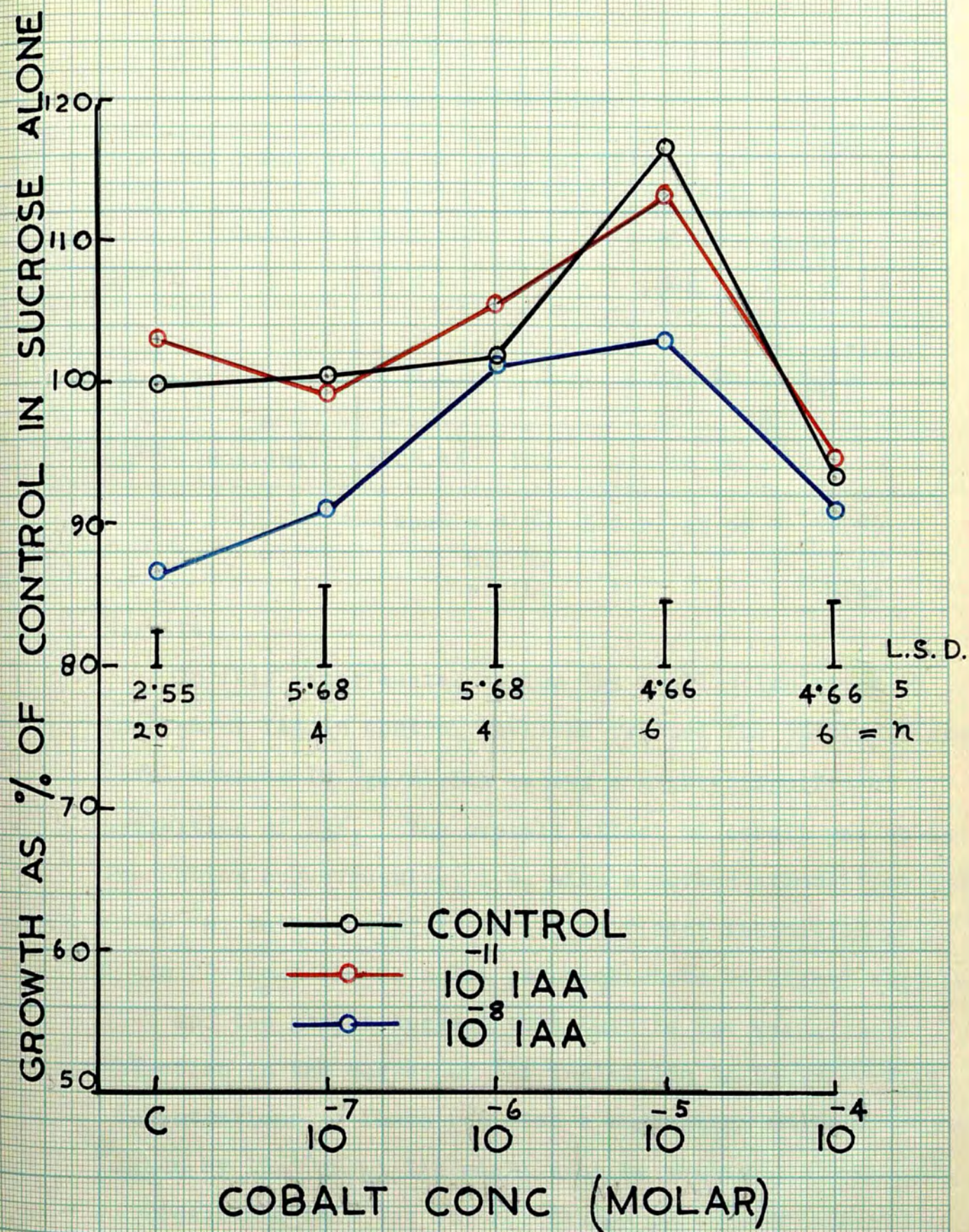




Table 29  
IAA-Cobalt Interaction

Nature of effect	Sources of variance	Sum of squares	Degrees of freedom	Mean sq. variance	P.
Main factors	Cobalt(Co)	3811.56	4	952.89	highly significant
	IAA(I)	3017.4	2	1508.7	"
Inter-action between pairs	Co X I	786.1	8	98.26	Significant at 1% level
Replication	Residual	1705.45	105	16.24	

when  $n = 20$ , L.S.D. at 5% =  $\pm 2.55$

$n = 4$ , " =  $\pm 5.68$

$n = 6$ , " =  $\pm 4.66$

#### Results:

In this experimental series the small stimulation by  $10^{-11}$  gm/ml IAA in control sections reached the 5% significance level whereas  $10^{-8}$  gm/ml IAA causes a highly significant inhibition of 13%. The overall effect of cobalt was as in the action curve results, i.e. with a marked stimulation peak at  $10^{-5}$  M. falling rapidly to a significant inhibition at  $10^{-4}$  M. Table 29 shows the Co x IAA interaction to be highly significant and the graphs suggest that this is due mainly to a reduction in the degree of IAA inhibition in the higher Co concentrations. There was also a suggestion that the stimulation by  $10^{-11}$  gm/ml IAA might also be abolished in the presence of cobalt and this was further tested by another experiment in which the time factor was taken into account.

(c) Cobalt and IAA interaction with time

The stimulating effect of cobalt is very significant at concentration  $10^{-5}$  M. This is of the order of 15%. Cobalt seems to antagonise auxin action. This interesting effect of cobalt needed further study. So further experiments were conducted using two stimulatory concentrations, i.e. one part in  $10^{11}$  and one part in  $10^{10}$  of IAA in combination with one concentration of  $10^{-5}$  M. of cobalt, which is also highly stimulatory. The plan of the experiments is shown below:

Table 30

Plan of Investigation

Conc.(molar) cobalt	Conc. (gm/ml.) IAA		
0	0	$10^{-11}$	$10^{-10}$
	6	6	6
$10^{-5}$	6	6	6

The length of the sections were measured at the intervals of (0-4), (4-7), (7-24) and (24-48) hours, under horizontal microscope. The sections were allowed to grow in agitator. The purpose of measuring the length of the sections is that at these stimulatory concentrations of IAA, the degree of stimulation of the sections is very high, and it is of the order of about 25%, specially in early hours. The rate of growth (extension) per hour was



Figure 14.

Graphs showing the interaction between Cobalt and B-indolylacetic acid (1AA) in the extension growth of excised pea root sections. The times noted above each set of graphs are the growth periods after excising from the parent root. The vertical lines represent the least significant differences at 5% probability level between any two pairs of means of growth rates in the series of concentration combinations against which they are placed. The figures against these lines record the numbers of replicate samples from which each mean of the series was calculated.



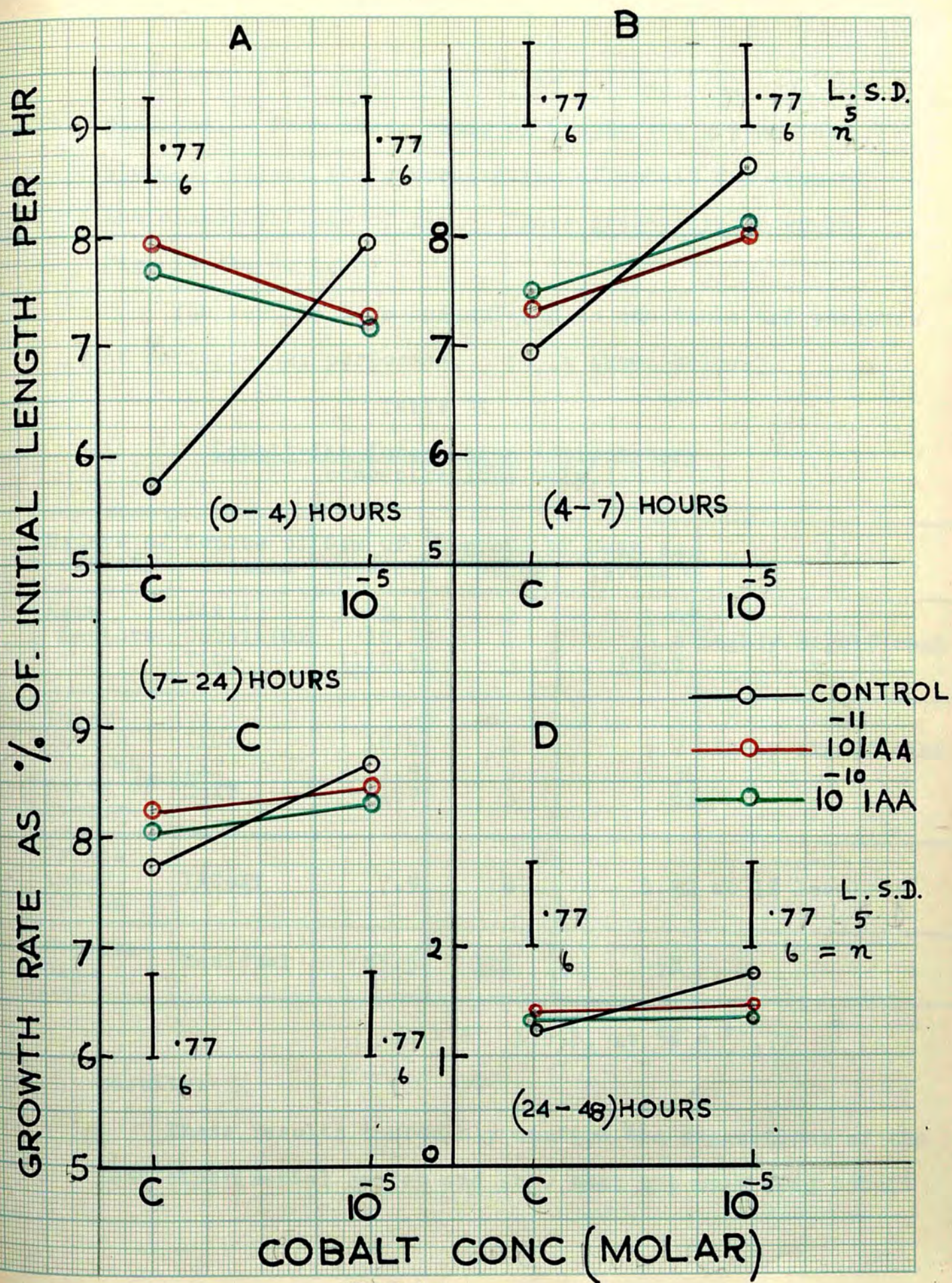


FIG 14



calculated as a percentage of the initial length of the sections. From these rates of growth the graphs were plotted against the concentration of cobalt for each of the concentrations of IAA. These graphs are shown in Fig. 14 (A, B, C, D).

The results were accumulated in one table and analysis of variance was performed. It is shown below:

Table 31

Cobalt-IAA Interaction (Stimulatory concentrations only.)

Nature of effects	Source of variance	Sum of squares	Degrees of freedom	Mean sq. variance	P.
Main factors	IAA(I) Cobalt(Co) Time	2.1 10.8 1118.5	2 1 3	1.05 10.8 372.8	- highly significant "
Inter-action between pairs of factors	IXCo IXT CoXT	10.4 1.9 1.9	2 6 3	5.2 .31 .63	highly significant - -
Triple inter-action	IXCoXT	7.9	6	1.32	at 1% level.
Replication	Residual	55.5	121	.458	

$$n = 6 \quad \text{L.S.D. at } 5\% = \sqrt{\phantom{x}} .77$$

#### Results:

These results thoroughly confirm the suggestions of the last experimental series and show that not only does cobalt

at stimulatory concentrations ( $10^{-5}$ M) completely abolish the stimulation of growth by both  $10^{-10}$ gm/ml and  $10^{-11}$ gm/ml IAA during the whole period of the growth of sections but that these two concentrations of auxin reduce significantly (at least in the first 7 hours of growth) the stimulation due to cobalt. This latter finding was foreshadowed by the previous experiment (see Fig. 13B). The highly significant triple interaction is seen in the graphs to be due to the disappearance of both IAA and cobalt effects and their interaction in the last stages of section extension.

#### Discussion:

The causes for this mutual antagonism between cobalt and IAA are obscure. Miller (1954) also obtained a marked stimulation by cobalt of the growth of etiolated stem sections, specially in presence of sugar and auxin. This observation led him to conclude that cobalt, like potassium, (Brown and Sutcliffe 1950) causes entry of more sugar into the stem tissue, which results in the promotion of growth. Same explanation may be applicable to the results noticed here, namely, that cobalt causes the entry



of sugar into root tissues leading to the stimulation of the growth of root sections. But this does not explain the antagonism between auxin and cobalt. It is, therefore, suggested that cobalt exerts a direct effect on the growth of root sections. Cobalt might probably impede the entry of auxin and vice versa, which results in mutual antagonism between them. This is merely a suggestion. If cobalt really increases the rate of sugar entry into the root sections, then we can expect cobalt to have a much smaller effect in stronger sucrose solutions. It would, therefore, be worth while to do some experiments with cobalt in presence of a stronger sucrose solution.

## (VI) Copper

### (a) The effect of copper alone (action curve)

Copper is a very toxic metal, one part in  $10^5$  molar solution is usually the optimum concentration for the heavy metals so far tested. But one part in  $10^5$  molar concentration of copper is very inhibitory. Concentration of copper  $10^{-4}$  molar is far more toxic and it kills the sections in 10 hours. [REDACTED] led. For this reason the effect of copper was investigated from concentrations as dilute as  $10^{-12}$  molar solution. But in the action curve there appear only those concentrations from  $10^{-9}$  to  $10^{-5}$  molar (Fig. 15 A). The action curve

Figure 15A

Graph showing the action curve of Copper on the growth of excised root sections over 48 hours. The vertical line represents the least significant difference at the 5% probability level between any two means. The figure against this line records the number of replicate samples from which each mean of the series was calculated.



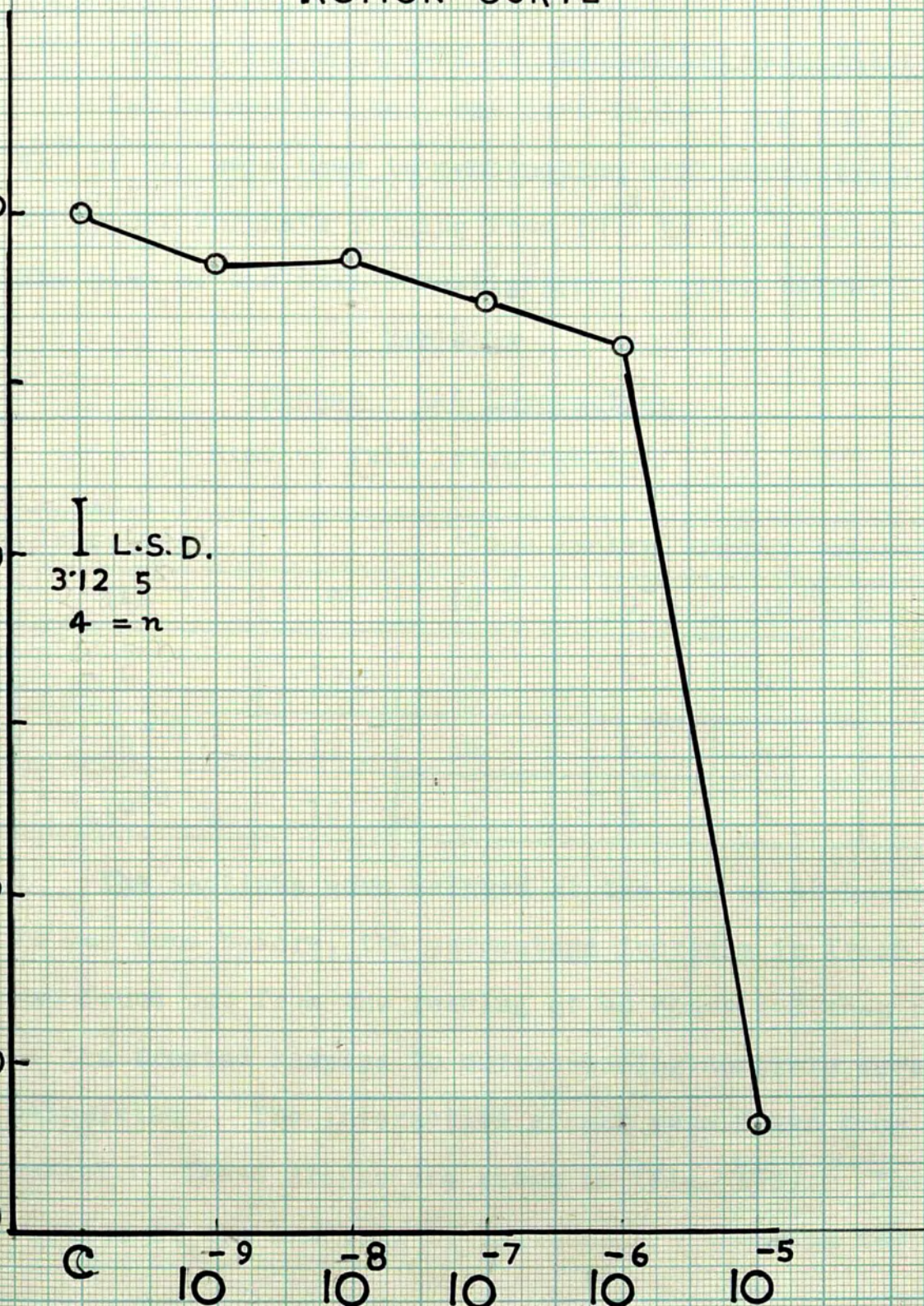
GROWTH AS % OF CONTROL IN SUCROSE ALONE

ACTION CURVE

L.S.D.  
3.12 5  
4 = n

COPPER CONC. (MOLAR)

FIG 15 A





does not indicate any stimulation with any concentrations. Concentration one part in  $10^{-5}$  molar causes an inhibition of the order of 50%.

(b) The effect of copper in combination with IAA

Two concentrations, one part in  $10^{11}$  and one part in  $10^8$  of IAA were used in combination with concentrations of copper sulphate ranging from  $10^{-8}$  to  $10^{-6}$ , molar solution. The plan of the full investigation is given below:

Table 32

Copper - IAA Interaction

Plan of full investigation.

Conc. (gm/ml.)	Conc. (molar) copper sulphate				
IAA	0	$10^{-8}$	$10^{-7}$	$10^{-6}$	
0	12	4	4	4	
$10^{-11}$	12	4	4	4	
$10^{-8}$	12	4	4	4	

The sections were weighed at the end of 48 hours. As it was done before, the percentage increase in fresh weight was found out from the reading at 48 hours and then ratios of the treated samples to corresponding controls were calculated. The interaction graphs were drawn from these ratios. These graphs are shown in (Fig. 15B). The results of the analysis of variance are shown on the next page:



Figure 15B

Graphs showing the effect of Copper on the growth response to B-indolylacetic acid (1AA) of root sections over 48 hours. The vertical lines represent the least significant differences at the 5% probability level between the means of the ratios to corresponding controls at the particular concentrations of Copper against which they are placed. The figures against these lines record the numbers of replicate samples from which each mean of the series was calculated.



GROWTH AS % OF CONTROL IN SUCROSE ALONE

—○— CONTROL  
<sup>-11</sup>  
 —○— 10<sup>-8</sup> IAA  
<sup>-8</sup>  
 —○— 10<sup>-8</sup> IAA

110  
100  
90  
80  
70  
60  
50

4.85  
12

8.4  
4

8.4  
4

8.4 5  
4 = n

L.S.D.

C

10<sup>-8</sup>

10<sup>-7</sup>

10<sup>-6</sup>

COPPER CONC (MOLAR)

FIG 15 B

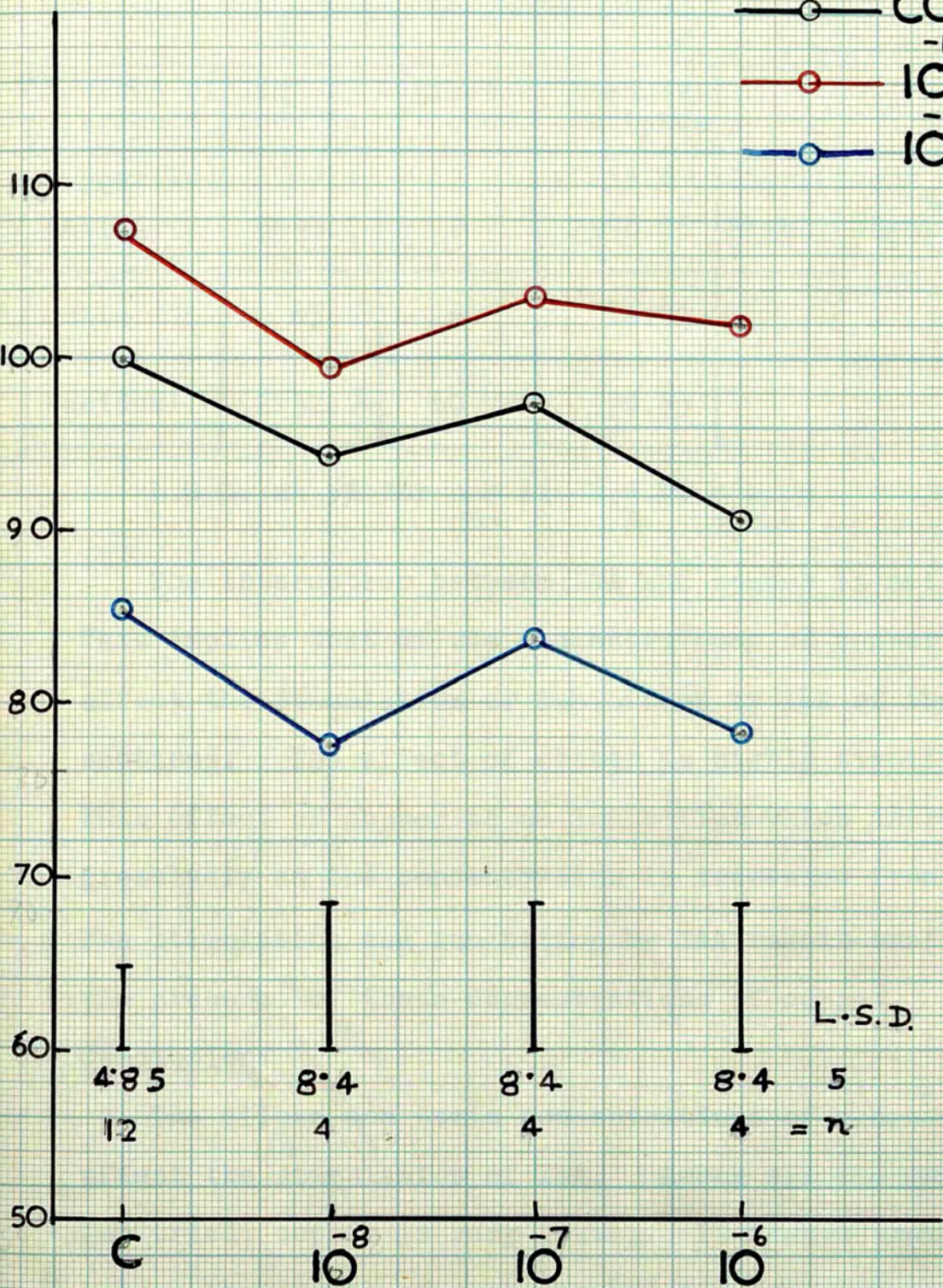




Table 33  
Copper-IAA Interaction

Sources of variance	Sum of squares	Degrees of freedom	Mean sq. variance	P.
Copper(Cu)	767.4	3	255.8	Significant at 1% level. Highly significant
IAA(I)	5996.1	2	2998.05	
CuXI	65	6	10.83	
Residual	2132.94	60	35.55	

L.S.D. at 5% for  $n = 12$ , L.S.D. = 4.85  
 $n = 4$  " = 8.4

The interaction between IAA and copper is not significant. This implies that dilute concentrations of copper do not affect the stimulation or inhibition by IAA of the growth of sections. The effect of copper is highly significant. This effect of copper is an inhibition which increases progressively as the concentration is increased from  $10^{-8}$ M- $10^{-6}$ M. The stimulation and inhibition by IAA remain unaffected by the presence of these concentrations of copper.

Conclusion:

The dilute non toxic concentrations of copper do not affect the stimulation of IAA. Among all the metals so far tried, copper is very toxic. It always tends to reduce the growth of sections but did not stimulate the growth of sections at any concentration however dilute

it may be.

General conclusion:

The results of the experiments carried out with metals, such as  $Mn^{++}$ ,  $Zn^{++}$ ,  $Co^{++}$ ,  $Ca^{++}$ ,  $Cu^{++}$ , and also boron indicate that the dilute concentrations of these metals which are likely to be present in ordinary distilled water or tap water as impurities do not modify the growth response of root sections to stimulatory concentrations of IAA.

It can, therefore, be suggested that the variations in response of sections to stimulatory concentrations of IAA observed by earlier workers of this laboratory are not caused by metallic impurities present in the ordinary distilled water.



## CHAPTER IV

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### THE INTERACTION OF AUXINS AND ANTIAUXINS IN THE STIMULATION OF ROOT GROWTH

#### Introduction:

The discovery of mutual antagonism of metabolites and antimetabolites, and between vitamins and vitamers prompted the auxin physiologists to study the interaction of auxins and antiauxins in the growth of Coleoptiles and roots.

Antiauxins, as the name suggests, are those compounds which counteract the effects of auxins in control of the growth of coleoptiles and roots. It is assumed that these anti-auxins compete with auxins for the growth centre where stimulation or inhibition (in root) is taking place. Therefore, antiauxins must have molecular structures similar to the molecular structure of auxins. It has been clearly shown that molecules of antiauxins are really a slight modification of the auxin molecules, such as 2,4-D. The major changes which convert an auxin molecule to an antiauxin one are as follows :-

- 1) Elimination of carboxyl group and retention of an unsubstituted ortho-position (e.g. 2,4-dichloro-anisole).
- 2) Blocking of both ortho-positions by substitution with retention of the carboxyl<sup>x</sup> group (e.g. 2,6-dichloro-phenoxy<sup>x</sup>-acetic acid).

3) Prevention of proper spatial relationships between carboxyl and reactive ortho groups, for example, bulky methyl substituents on the  $\alpha$ -carbon (steric hindrance) (e.g. p-chloro-phenoxy-iso-butyric acid (P.C.I.B.) ).

The well known antiauxins are:

2,4-dichloro-anisole, (D.C.A.) Bonner, 1949; 2,3,5-tri-iodo-benzoic acid, (T.I.B.A.) Galston, 1947; Maleic hydrazide, (M.H.) Leopold and Klein, 1951;  $\alpha$ (1-naphthyl-methyl-sulphide) propionic acid (N.M.S.P.) Åberg, 1950; p-chloro-phenoxy-isobutyric acid (P.C.I.B.) Burström, 1950; 4-chloro-3-nitro-benzoic acid (C.N.B.) Minarik et al. 1952.

An account of the work on the auxin and antiauxins, as reported by many authors, in growth of root, is given in the introduction (p. 6 ).

Åberg (1950, 1951) working with N.M.S.P., showed that N.M.S.P. alone caused stimulation of flax root about 15 to 20%. A range of N.M.S.P. concentrations ( $10^{-7}$  -  $2 \times 10^{-5}$  M) used in combination with 2,4-D, IAA and N.A.A. releases the inhibition of root by these auxins. These auxins also in turn antagonise the stimulation caused by N.M.S.P. alone.

He assumed that these auxins and antiauxins compete with each other for the growth centre, where this inhibition is taking place. He is of the opinion that antiauxins which have low 'activity' displace the highly active auxins at these centres, thus relieving the auxin inhibition of root growth.



Antiauxins, therefore, have ~~high~~ "affinity" and low or zero "activity". On this basis weak auxins may also antagonise strong ones.

Burström(1950) studied critically the activity of P.C.I.B. on the growth of intact wheat roots. He found that P.C.I.B. stimulates the growth of root about 60% in first two days. IAA at ( $10^{-8}M$ ) antagonises the stimulation by P.C.I.B., and P.C.I.B. at ( $10^{-6}M$ ) also alleviates the IAA-inhibition at ( $10^{-6}M$ ). He thus established the antagonism between auxin (IAA) and P.C.I.B.

Minarik et al (1952) similarly reported that 4-fluoro-3-nitro-benzoic acid counteracts the ~~2,4-D~~ inhibition of cucumber roots. It is suggested that these antiauxins cause the stimulation of root growth by antagonising the native auxin of root which is in supra-optimal concentration. These results clearly suggest that there is a clear-cut antagonism between auxins and antiauxins in the inhibition of root growth.

Such an approach to the study of auxin and antiauxin on the inhibition of root growth sounds very rational, had it not been for the fact that both auxin<sup>(IAA)</sup> and antiauxins in low concentration, cause stimulation of the growth of root.

This observation of the identical action of auxin and antiauxins in the stimulation of root growth led Audus and Shipton (1952) to postulate a theory that both auxins and

antiauxins have virtually the same action on the growth of root. [redacted] They suggested that both auxins and antiauxins stimulate growth by antagonising a natural inhibitor present in the extending cells. The degree of stimulation by the compound (auxin or antiauxin) would be determined by the extent to which the normal growth is being suppressed by this inhibitor and the effectiveness of the compound as its antagonist. At much higher concentrations both compounds would themselves cause inhibition, presumably at different growth centres.

A study of the interaction of auxin and antiauxins in the stimulation of the growth of pea root sections, has been carried out in the hope that results of this interaction will throw some light on the theory postulated by Audus and Shipton (1952).

In this thesis will be described the results of a series of experiments on the extension growth of root sections carried out with two stimulatory concentrations (one part in  $10^{11}$  and  $10^{10}$ ), used in combination with a range of concentrations of the antiauxins mentioned below:

The following antiauxin compounds were placed at my disposal by the courtesy of the persons mentioned:

- 1) 4-chloro-3-nitro-benzoic acid, (C.N.B.) ( $C_6H_3Cl.No_2$  COOH). (Professor Audus, Bedford College).



- 2)  $\alpha$ -(1-naphthyl-methyl-sulphide)propionic acid (N.M.S.P.)  
 $(C_{10}H_7CH_2.S.CH.(CH_3)COOH)$ . (Professor Arne Fredga,  
 Uppsala, Sweden and Pal Chemical).
- 3) *p*-chloro-phenoxy-iso-butyric acid (P.C.I.B.)  
 $(C_6H_4.Cl.O.C.(CH_3)_2.COOH)$ . (Professor R. L. Wain,  
 Wye College).
- 4) 1-(Naphthyl-methyl-sulphide-acetic acid) (N.M.S.A.)  
 $(C_{10}H_7CH_2.S.CH_2COOH)$ . (Dr. Börge Åberg, Royal  
 Agricultural College, Sweden).

#### 4. The effects of growth substances acting alone

##### (I) Concentration-response curves of B-indolyl-acetic acid (IAA) on the growth of pea root sections

Pea root sections of 2-m.m. long excised from extending zone of the radicles of 2-day old seedlings were treated with a range of concentrations (one part in  $10^{14}$ - $10^7$ ) of IAA in  $\frac{1}{2}\%$  sucrose solution. The growth of the sections was observed for a period of 48 hours. During the growth period the petri-dishes containing the growing sections were rocked through an angle of  $45^\circ$  from the horizontal on an electrically-driven device. At regular intervals of (0-4), (4-7), (7-24) and (24-48) hours the samples were removed from the agitator and the lengths of the sections were measured to the nearest 0.05 m.m. Under a travelling

microscope. Sterile petri-dishes and instruments were used to ensure sterility. The samples were then returned to the shaker.

The plan of the experiments is shown below:

Table 34

Action curve of IAA in the extension growth  
of pea root sections

Plan of Experiment

Number of samples of 10 Sections

Expt.	Conc. (gm/ml.) IAA								
	0	$10^{-14}$	$10^{-13}$	$10^{-12}$	$10^{-11}$	$10^{-10}$	$10^{-9}$	$10^{-8}$	$10^{-7}$
E1	2	2	2	2	2	2	2	2	2
E2	2	2	2	2	2	2	2	2	2
E3	2	2	2	2	2	X	X	X	X

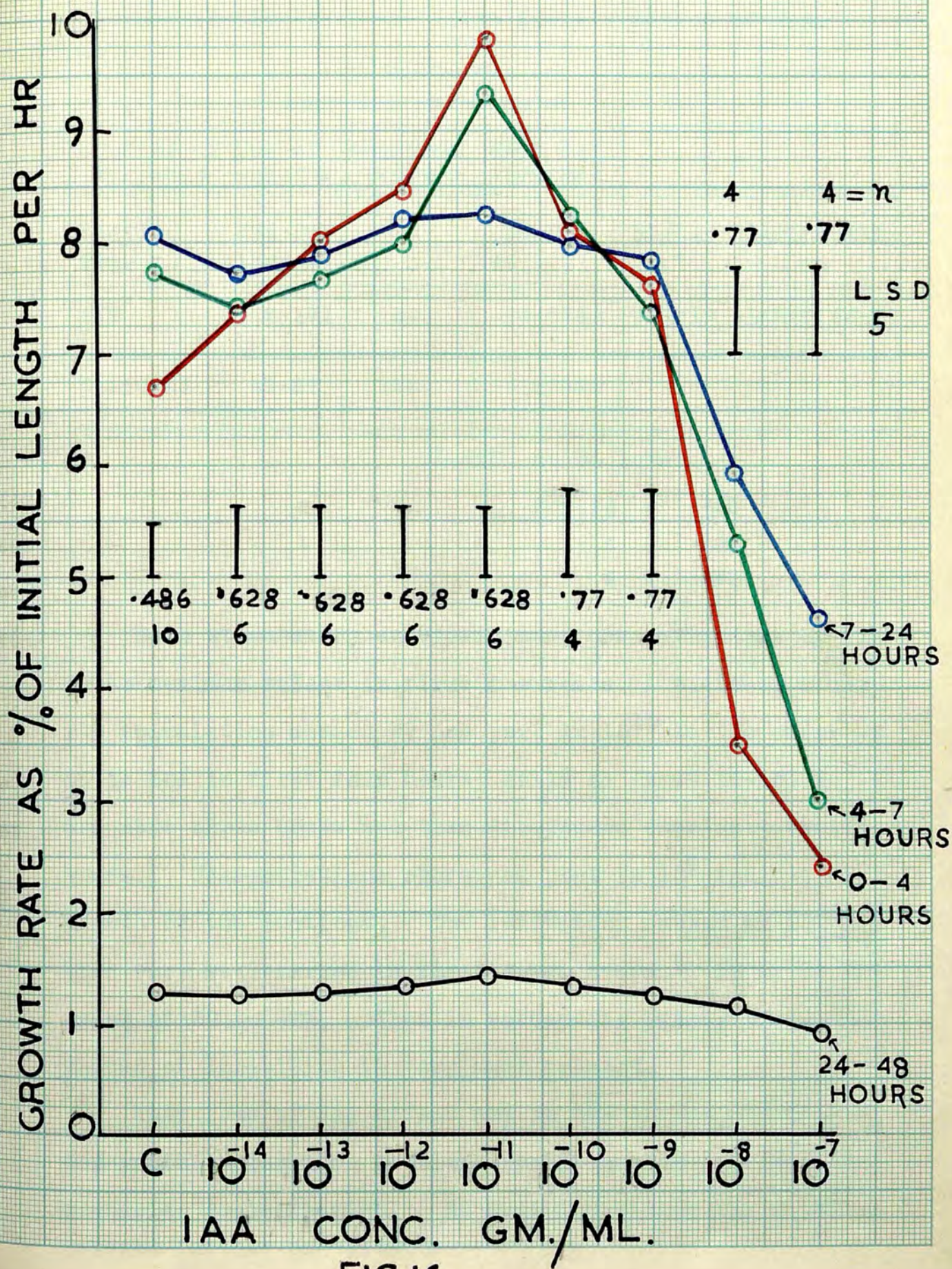
From this data the rate of growth (extension) per hour was calculated as a percentage of the initial size of the sections. The graphs which are drawn at different intervals of time against IAA concentrations are shown in (Fig. 16).



Figure 16

Concentration-response curves for the action of B-indolyl-acetic acid (IAA) on the extension growth of excised pea root sections showing the <sup>Changes</sup>~~changes~~ in response taking place during the course of the extension. The vertical lines represent the least significant differences at the 5% probability level between growth rates <sup>at</sup>~~and~~ the relevant concentrations. The figures below the lines record the numbers of replicate samples from which each mean was calculated.







## Concentration-response curve for the action of IAA.

Table 35  
Analysis of Variance

Sources of variance	Sum of squares	Degrees of freedom	Mean sq. variance	P.
IAA(I)	272.393	8	34.05	highly significant.
Time (T)	1384.41	3	461.47	" "
IXT	112.171	24	4.674	highly significant.
Residual	48.87	164	.298	

L.S.D. at 5% level.

for 10 = .486

6 = .628

4 = .770

Both stimulatory and inhibitory concentrations of IAA cause maximum effect in first few hours, i.e. (0-7) hours. The stimulation caused by one part in  $10^{11}$  is the order of 20 to 30% in first four hours, falls to about 10-15% in the next three hours and then disappears over next twenty hours. Concentrations above one part in  $10^9$  inhibit growth of the sections. The inhibition caused by  $10^{-8}$  <sup>gm/ml</sup> is also very well-marked in first seven hours. It is of the order of 30 to 40%. Then in succeeding twenty hours the amount of inhibition is very much reduced.

This loss of IAA effect may be due to inactivation of the auxin by the root sections, or it may be also due to bacterial contamination. No definite answer can be given without further experiments.

(II) Concentration-response curve of N.M.S.P.

A range of concentrations of N.M.S.P. used in determining its action curve was from (one part in  $10^{10}$  to one part in  $10^3$ .) The sections were grown in the agitator for a period of 48 hours.

The plan of the experiment is shown below:

Table 36

Action curve of N.M.S.P. in the extension on growth of pea root sections

Plan of Experiments

Number of Samples

Expt.	N.M.S.P. concentrations (gm/ml .)									
	0	$\frac{10^{-10}}{10}$	$\frac{10^{-9}}{10}$	$\frac{10^{-8}}{10}$	$\frac{10^{-7}}{10}$	$\frac{10^{-6}}{10}$	$\frac{10^{-5}}{10}$	$\frac{10^{-4}}{10}$	$\frac{10^{-4}}{5 \times 10}$	$\frac{10^{-3}}{10}$
E1	2	2	2	2	2	2	2	2	2	2
E2	2	2	2	2	2	2	2	2	2	2
E3	2	2	2	2	2	2	2	2	2	2

The sections were allowed to grow for a period of 48 hours. After that time, the petri-dishes were removed from the agitator and the increase in fresh weight was measured. The ratios of the growths of the treated samples to those of the corresponding controls were calculated. The concentration-growth response curve was drawn from the average of the ratios against various N.M.S.P. concentrations. It is reproduced in Fig. 17.



Figure 17

Concentration-response curve for the action of  $\alpha$ -(1-naphthyl-methyl-sulphide)-propionic acid (N.M.S.P.) in the extension growth of excised pea root sections from overall growth in 48 hours. The vertical line represents the least significant difference at 5% probability level between any two means. The figure below the line records the number of replicate samples from which each mean of the ratios was calculated.

$E_1$ ,  $E_2$ , and  $E_3$  refer to three separate experimental series.



GROWTH AS % OF CONTROL IN SUCROSE

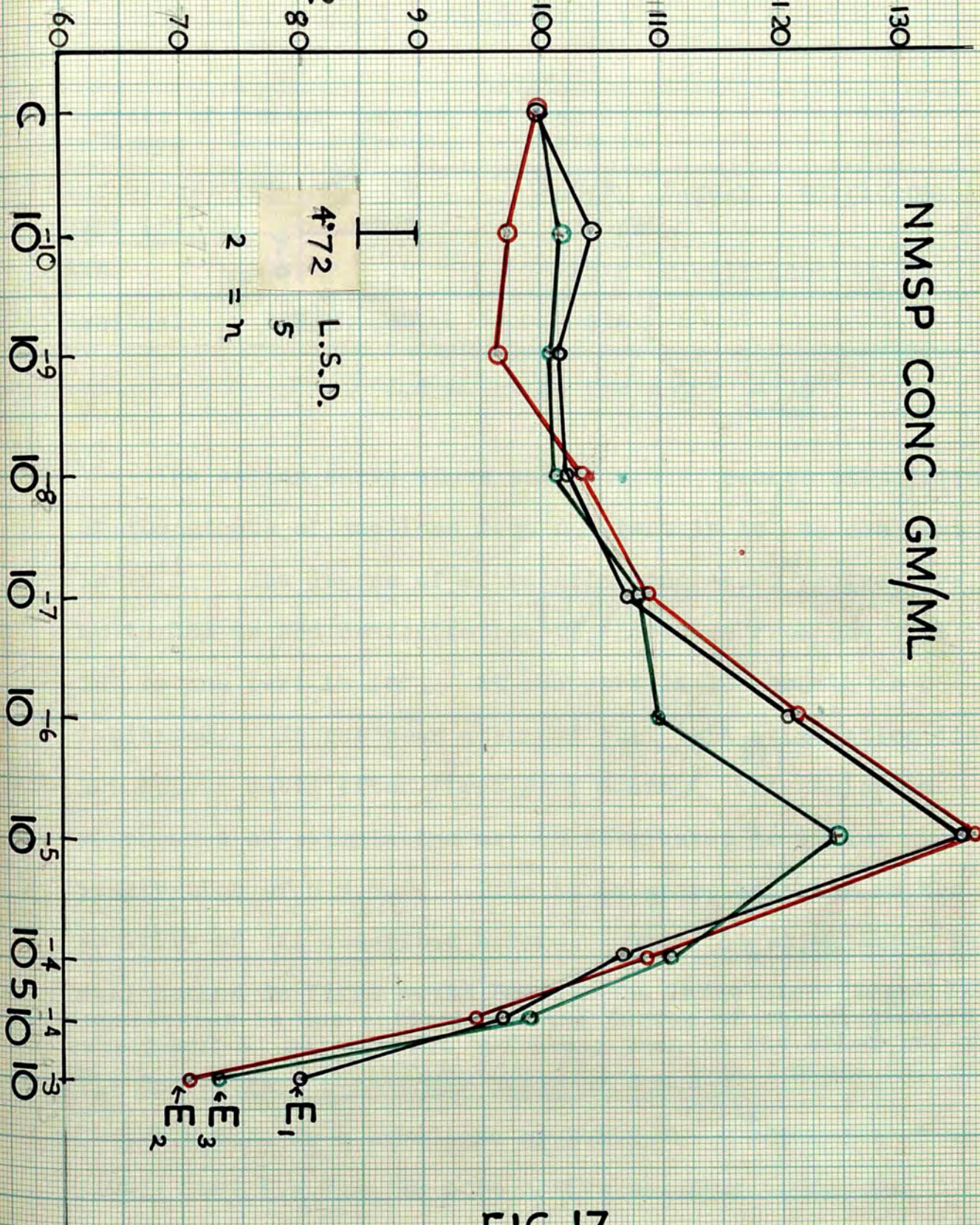


FIG 17



Concentration-response curve for the action of N.M.S.P. on the overall growth (at 48 hours) root sections.

Table 37

Sources of variance	Sum of Squares	Degrees of Freedom	Mean Sq. variance	P.
N.M.S.P.(N)	11594.0	9	1288.2	Highly significant.
Expt.(E)	46.7	2	23.35	
EXN	429.1	18	23.8	Significant. at 1%.
Residual	168.0	30	5.6	

L.S.D. at 5% for  $n=2$  = 4.72

The action curve shows that N.M.S.P. causes stimulation of the growth of sections from one part in  $10^8$  to one part in  $10^{11}$ . The degree of stimulation increases progressively up to 30% as the concentration of N.M.S.P. increases from one part in  $10^8$  to one part in  $10^5$ . At one part in  $10^3$  growth is inhibited. It has been observed that this stimulation did not appear until after first four hours. (See Fig. 22) This delay in appearance of stimulation may be attributed to a slow penetration of N.M.S.P. This is in sharp contrast to the activity of IAA described before. With IAA, stimulation is well-marked in first seven hours. The large significant EXN interaction is due entirely to the degree of stimulation in experiment  $E_3$  being about 30% lower than in the other two experiments ( $E_1$  and  $E_2$ ).

(III) Concentration-response curve of N.M.S.A.

N.M.S.A. is a homologue of N.M.S.P. Aberg has shown (1951) that N.M.S.A. is more active but less toxic than N.M.S.P.

Figure 18 A

Graph showing the concentration-response curve for the action of 1 -naphthyl-methyl - sulphide acetic acid (N.M.S.A.) in the extension growth of pea root sections from overall growth in 48 hours. The vertical line shows the least significant difference at 5% probability level between any two means. The figure below the line records the number of replicate samples from which each mean of the ratios was calculated.



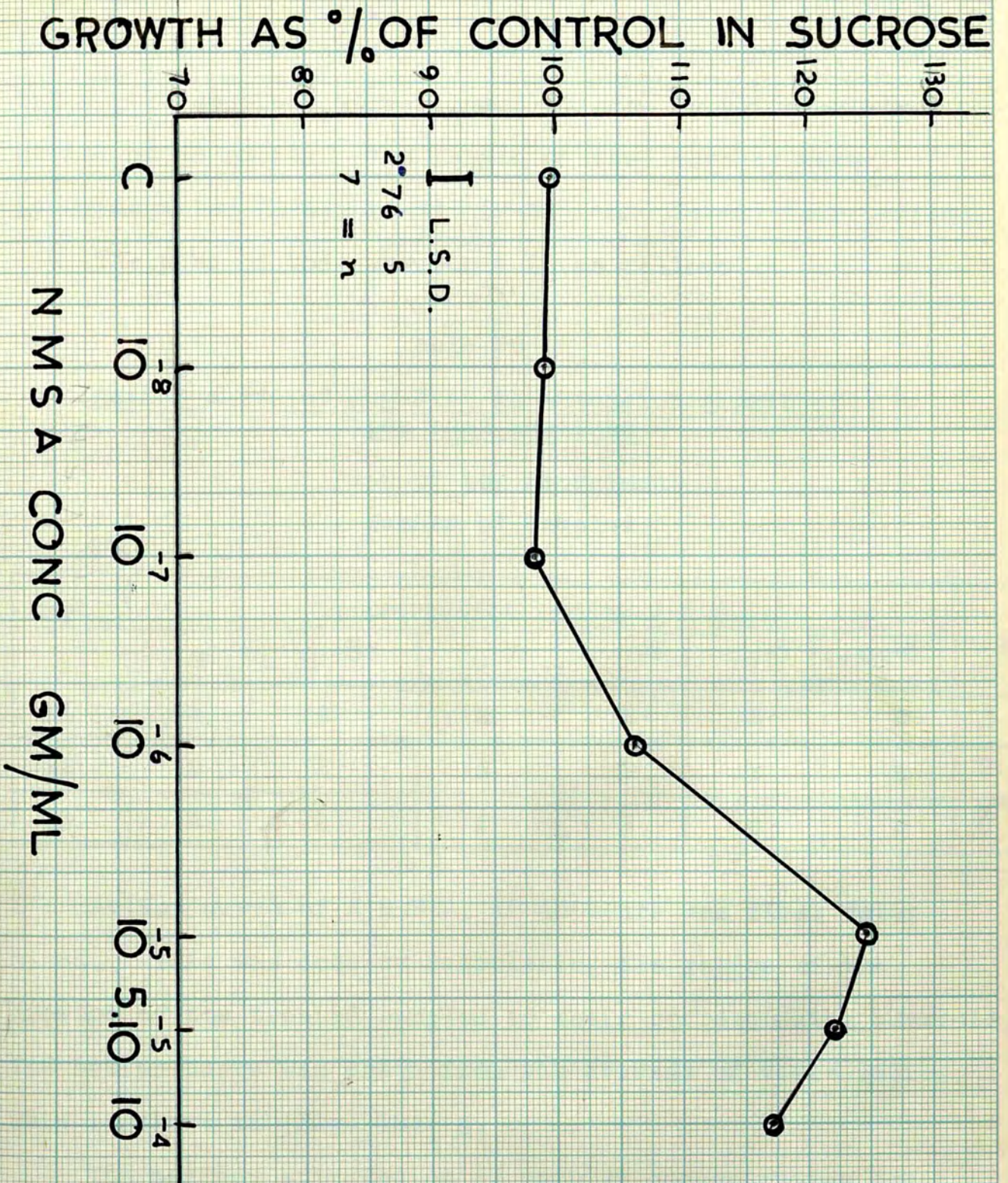


FIG 18 A



The excised root sections were treated with one part in  $10^8$  to one part in  $10^4$  concentrations of N.M.S.A. As before, the sections were allowed to grow in agitator for a period of 48 hours. The length of the sections were measured after (0-4), (4-7), (7-24) and (24-48) hour periods.

The plan of the experiment is as follows :

Table 38

Action curve of N.M.S.A. in extension growth of  
pea root sections

Plan of the Experiment

Number of Samples


Expt.	Concentration (gm/ml.) N.M.S.A.						
	0	$10^{-8}$	$10^{-7}$	$10^{-6}$	$10^{-5}$	$5 \times 10^{-5}$	$10^{-4}$
E1	2	2	2	2	2	2	2
E2	2	2	2	2	2	2	2
E3	2	2	2	2	2	2	2

Action curve of N.M.S.A. at 48 hours was first determined. This was done by calculating the ratios of the growths of the treated samples to their corresponding controls. The action curve was drawn from the average of these ratios. This is shown in (Fig. 18). A.

The maximum stimulation is seen at concentration one part in  $10^5$ . It is of the order of 24%. The stimulation starts from concentration as dilute as one part in  $10^7$ . A progressive rise in degree of stimulation is shown as the



Fig 18 B & C

The graphs showing the concentration-response curves for the action of 1 - nampthyl-methyl-sulphide acetic acid (N.M.S.A) on the extension growth root sections showing the changes in response taking place during course of extension. The vertical lines represent the least significant differences at  the 5% probability level between any two means. The figures below the lines record the number of replicate samples from which each mean of the growth rates was calculated.



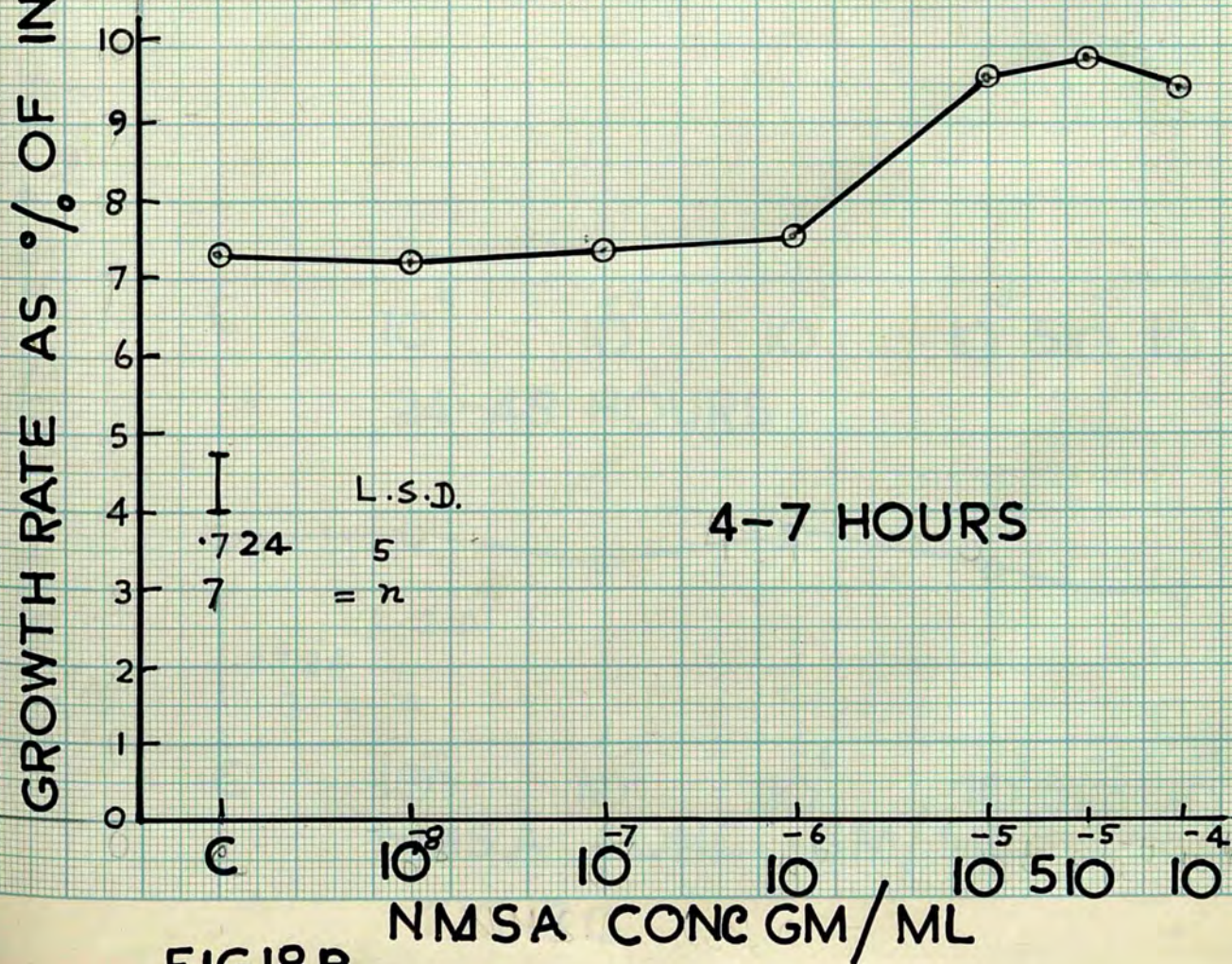
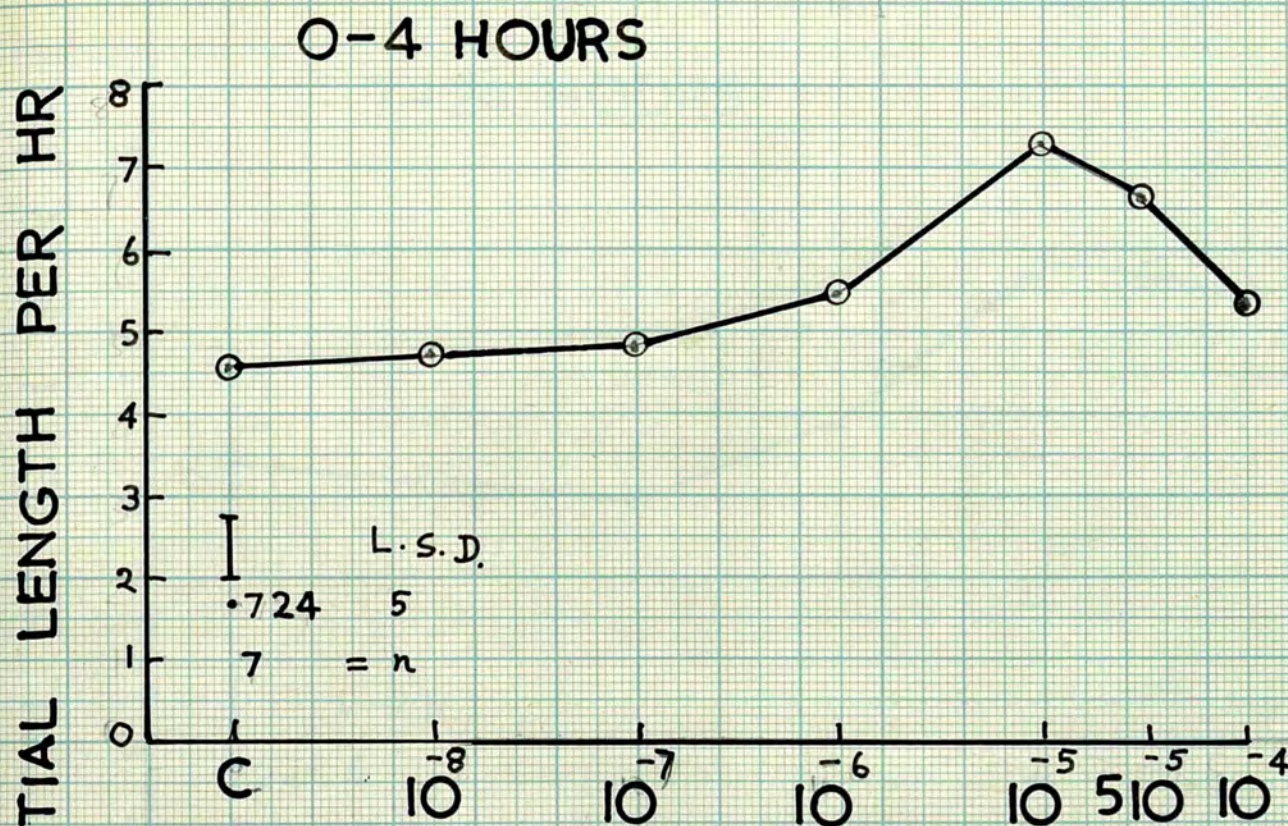


FIG 18 B



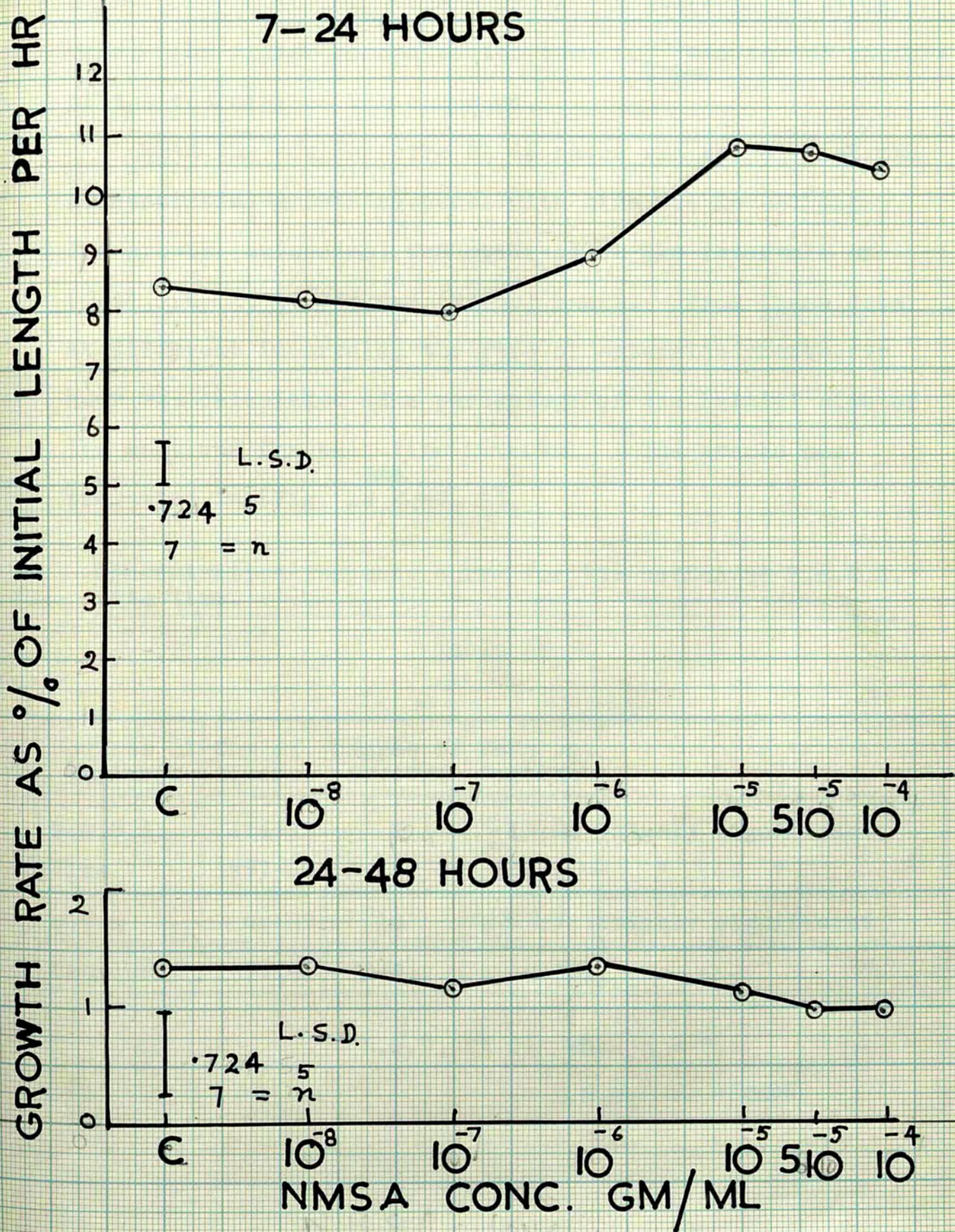


FIG 18 C



concentration increases from one part in  $10^7$  to  $10^5$  or  $5 \times 10^5$ . Then at  $10^{-4}$  <sup>gm/ml</sup> there is slight decrease in stimulation. No higher concentration than  $10^{-4}$  <sup>gm/ml</sup> was tested because of the scarcity of the compound.

When the action curve at 48 hours was established the concentration-growth response with time was investigated. The growth (extension) was observed at the intervals of (0-4), (4-7), (7-24) and (24-48) hours. From these data at different intervals, the rate of extension growth per hour was calculated as a percentage of the initial size of the sections for each time interval. The graphs are presented in (Fig. 18 <sup>HIA</sup> B, C, ☐).

Concentration-response curve for the action of N.M.S.A. with time.

Table 39

Sources of variance	Sum of squares	Degree of freedom	Mean sq. variance	P.
N.M.S.A.	114.13	6	19.02	highly significant.
Time	1977.93	3	659.31	" "
T X N	62.87	18	3.49	highly significant.
Residual	77.48	168	0.461	

L.S.D. at 5% when  $n = 7$ ,

L.S.D. = 0.724



Unlike [redacted] N.M.S.P., the growth response to N.M.S.A. is immediate. Even ~~in~~ the first four hours there is a very big stimulation. This stimulation persisted over the period of 24 hours, but after 24 hours the stimulation disappears. This large stimulation in the first (0-7) hours suggests that the rate of penetration of N.M.S.A. into the root sections is very rapid.

So far as toxicity is concerned, N.M.S.A. is less toxic than N.M.S.P. With N.M.S.P., inhibition starts from  $2 \times 10^{-4}$  gm/ml, whereas N.M.S.A. causes significant stimulation even at one part in  $10^4$ .

#### (IV) Concentration-response curve of P.C.I.B.

The action curve of P.C.I.B. was determined by using a range of concentrations from one part in  $10^8$  to one part in  $10^5$ . The effect of one part in  $10^4$  was also studied, but it was found very toxic, the sections being killed after 12 hours.

The plan of the experiments is given below:

Table 40  
P.C.I.B. Action Curve  
Plan of Experiment

Expt.	Conc. (gm/ml.) P.C.I.B.				
	0	$10^{-8}$	$10^{-7}$	$10^{-6}$	$10^{-5}$
E1	3	3	3	3	3
E2	3	3	3	3	3
E3	3	3	3	3	3

Figure 19

Concentration-response curve for the action of p-chlorophenoxy-iso-butyric acid (P.C.I.B.) in the extension growth of root sections from overall growth in 48 hours. The vertical line represents the least significant difference at 5% probability level between any two means. The figure below the line records the number of replicate samples from which each mean of the ratios was calculated.



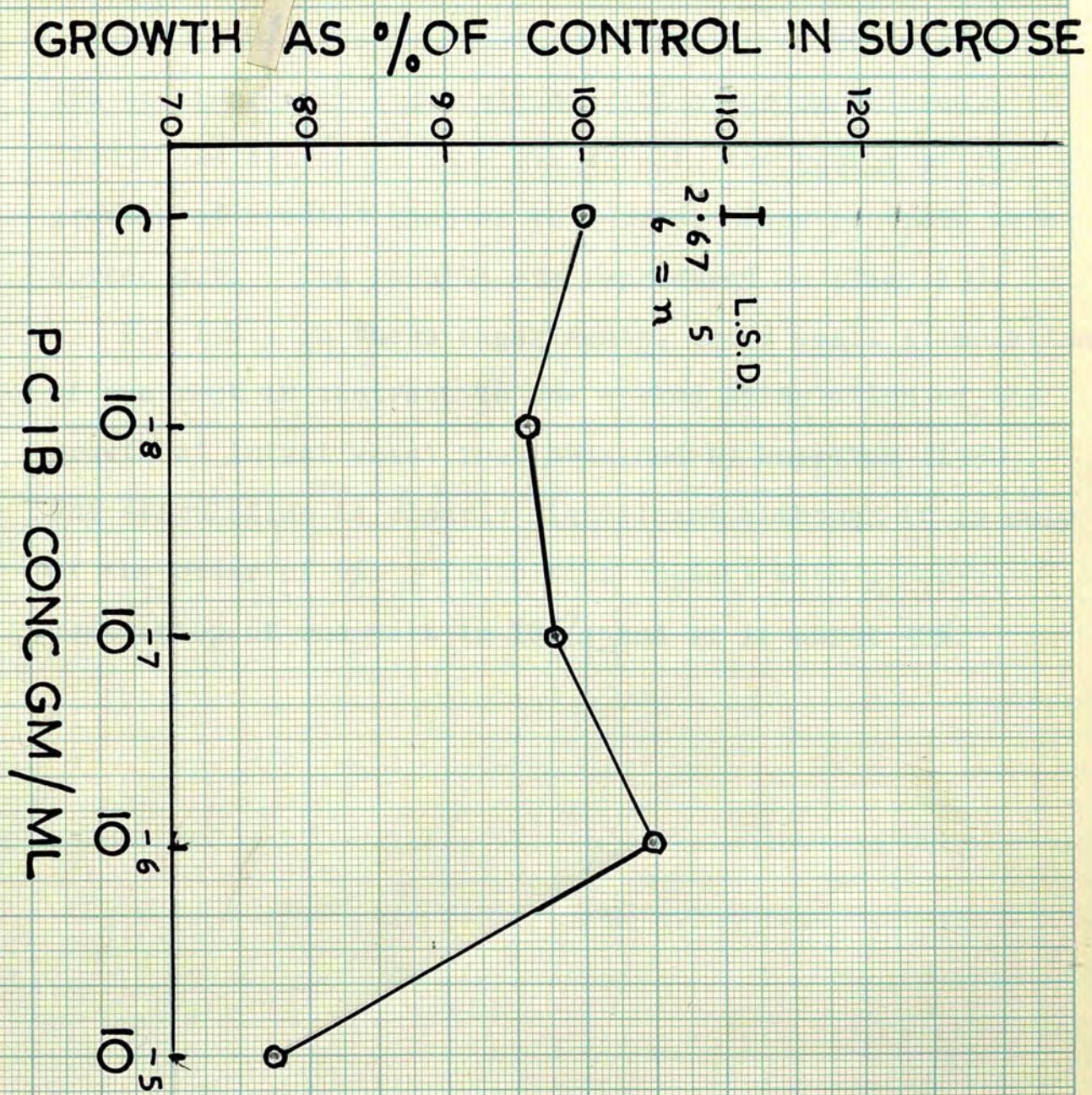


FIG 19



The growth was followed for a period of 48 hours. The sections were then weighed with a Torson balance. From these increases in fresh weight, the ratios of the growths of treated samples to corresponding controls were worked out. The results are reproduced in (Fig. 19).

P.C.I.B., in these experiments, shows small stimulation of pea root in contrast to the marked stimulation of the growth of intact wheat root obtained by Burstrom (1950). The stimulation is clearly significant although it is only of the order of 5%. Inhibition appears with a concentration of one part in  $10^{+5}_{gm/ml}$  and it is about 30%.



Figure 20A

The concentration-response curve for the action of 4-chloro, 3-nitrobenzoic acid (C.N.B.) in the extension growth of pea root sections from overall growth in 48 hours. The vertical line shows the least significant difference at the 5% probability level between any two means. The figure below this line records the number of replicate samples from which each mean was calculated.



# GROWTH AS % OF CONTROL IN SUCROSE

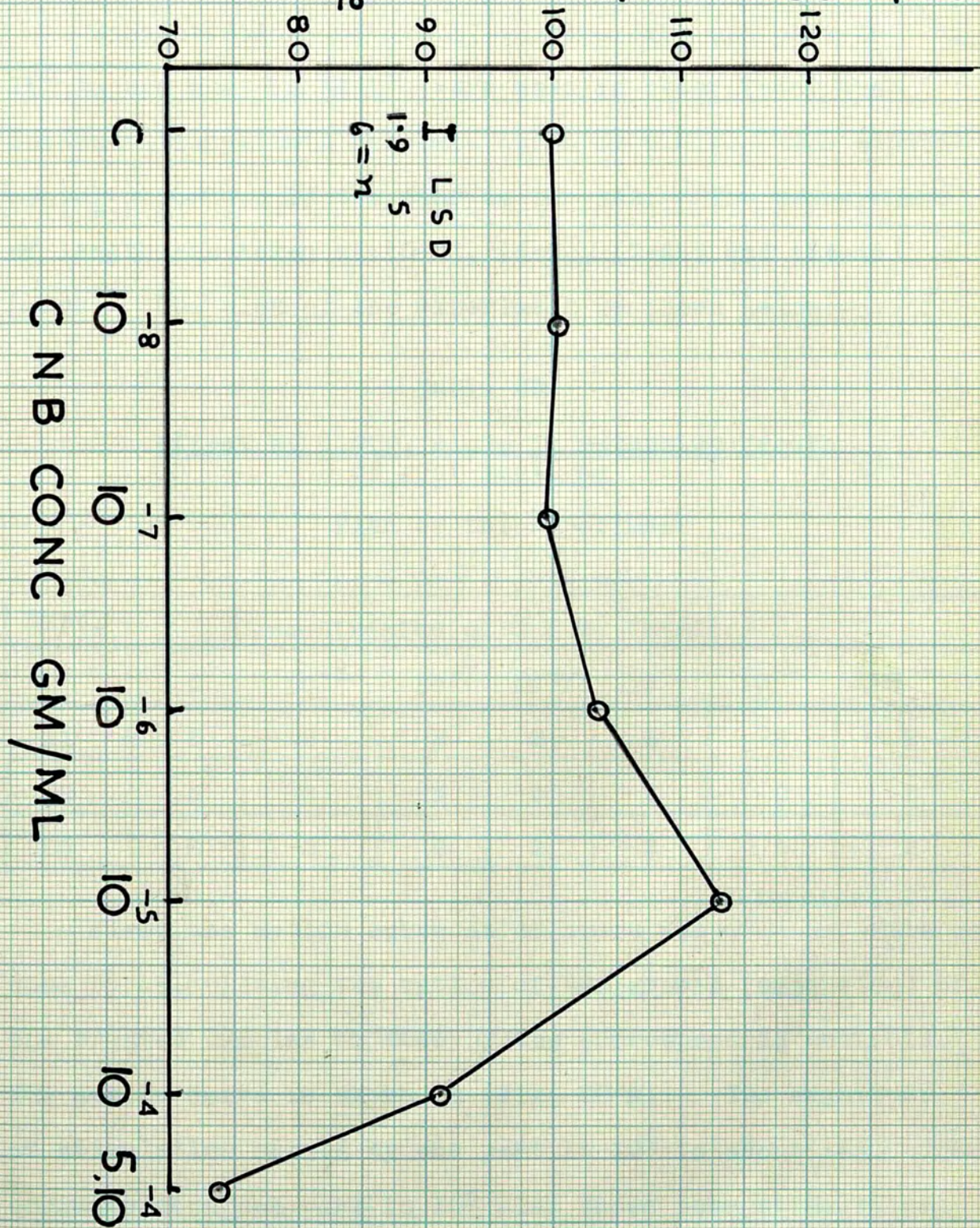


FIG 20 A



(V) Concentration-response curve of C.N.B.

Concentrations of C.N.B. ranging from one part in  $10^8$  to one part in  $5 \times 10^4$ , were used in determining the concentration-growth response curve of C.N.B. The experimental procedure is the same as for IAA. The measurement of length was taken at (0-4), (4-7), (7-24) and (24-48) hours. The plan of the experiment is shown below:

Table 42

Effect of C.N.B. alone in extension growth  
of root sections

## Plan of the Experiment

Expt.	Concentration of C.N.B. (gm/ml.)						
	C	$10^{-8}$	$10^{-7}$	$10^{-6}$	$10^{-5}$	$10^{-4}$	$5 \times 10^{-4}$
E1	2	2	2	2	2	2	2
E2	2	2	2	2	2	2	2
E3	2	2	2	2	2	2	2

determined.

First the action curve at 48 hours was [redacted]. This was done by calculating the ratios of the growth (extension) of the treated samples to those of the corresponding controls. The action curve is shown in (Fig. 20 **A**). The magnitude of stimulation caused by one part in  $10^5$  is about 14%. The concentration one part in  $10^6$  also shows some stimulation. The concentrations one part in  $10^4$  and  $5 \times 10^4$  are inhibitory.

Figure 20 B & C.

The graphs showing the concentration-response curves for the action of 4-chloro, 3-nitro-benzoic acid (C.N.B.) in the extension growth of pea root sections showing the changes in response taking place during extension. The vertical lines represent the least significant difference at 5% probability level between any two means. The figure below the lines records the number of replicate samples from which each mean of the growth rates was calculated.



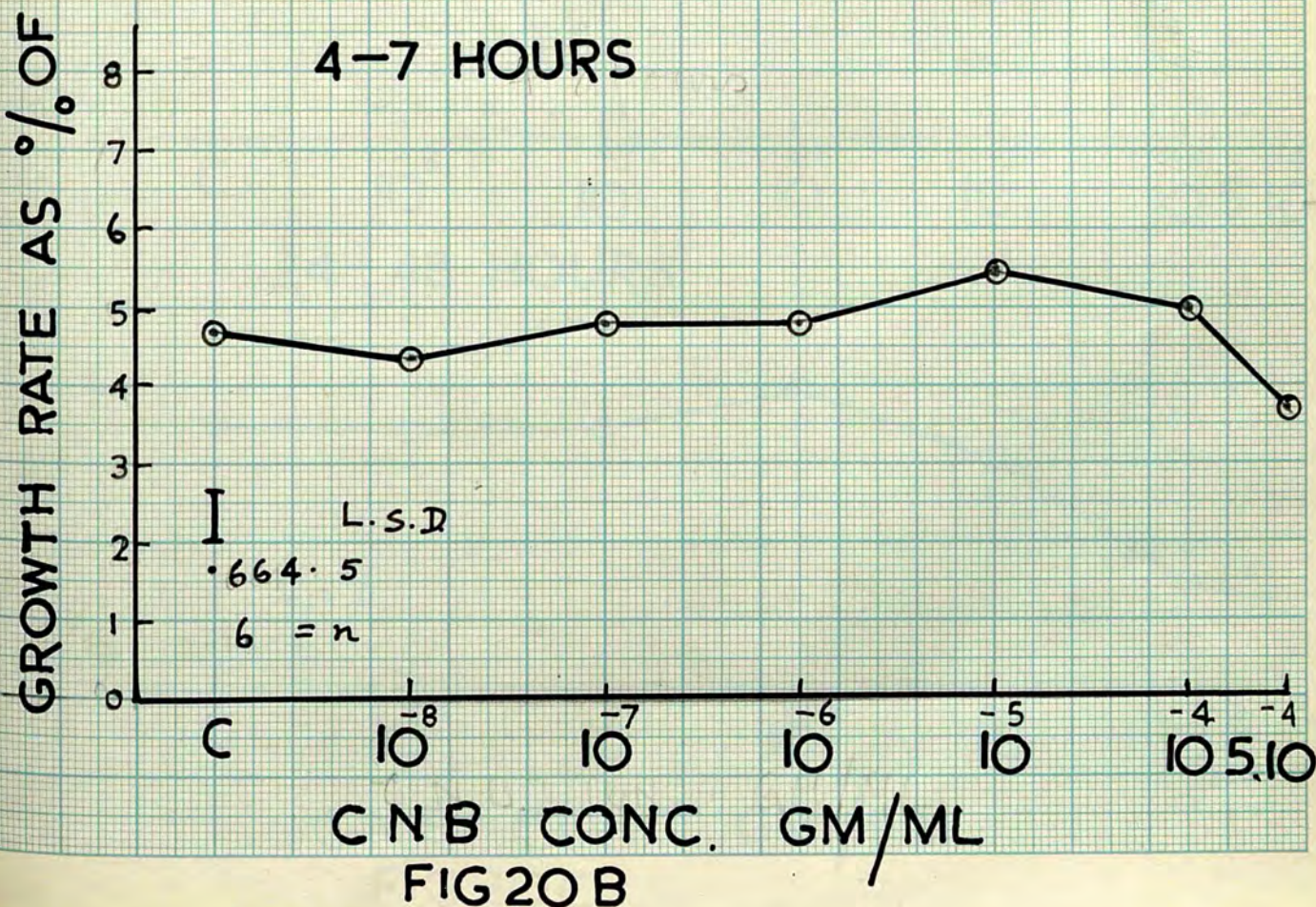
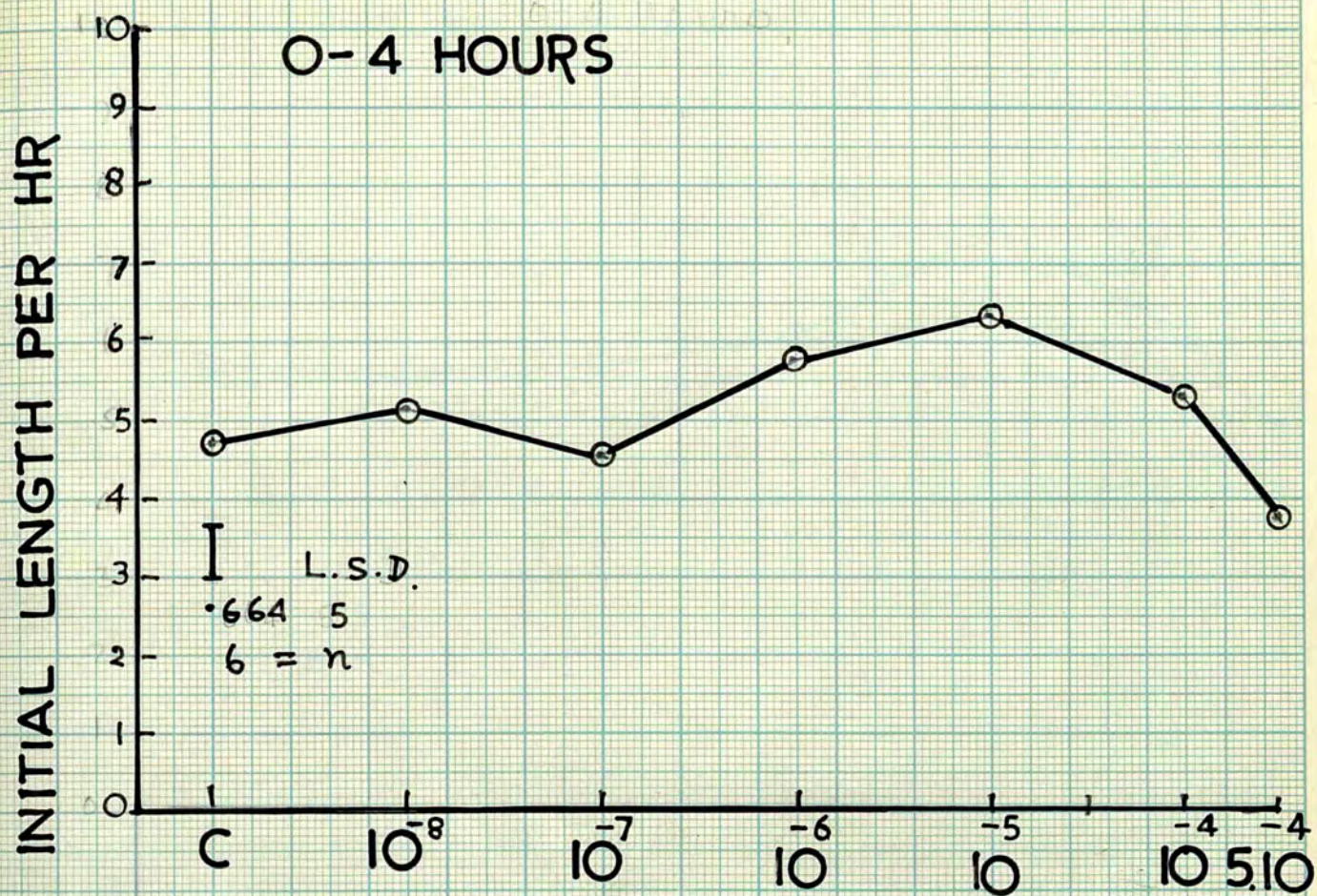
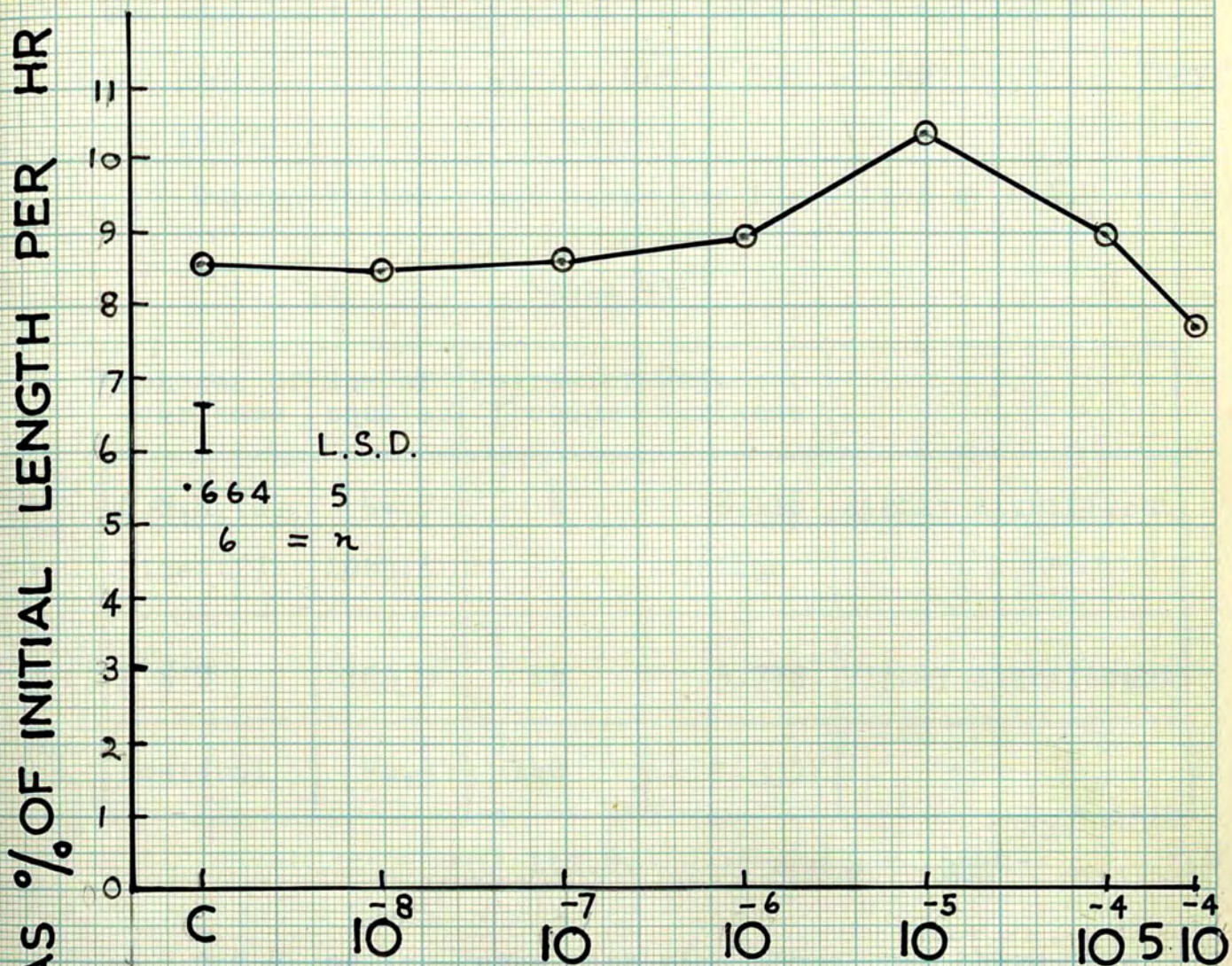


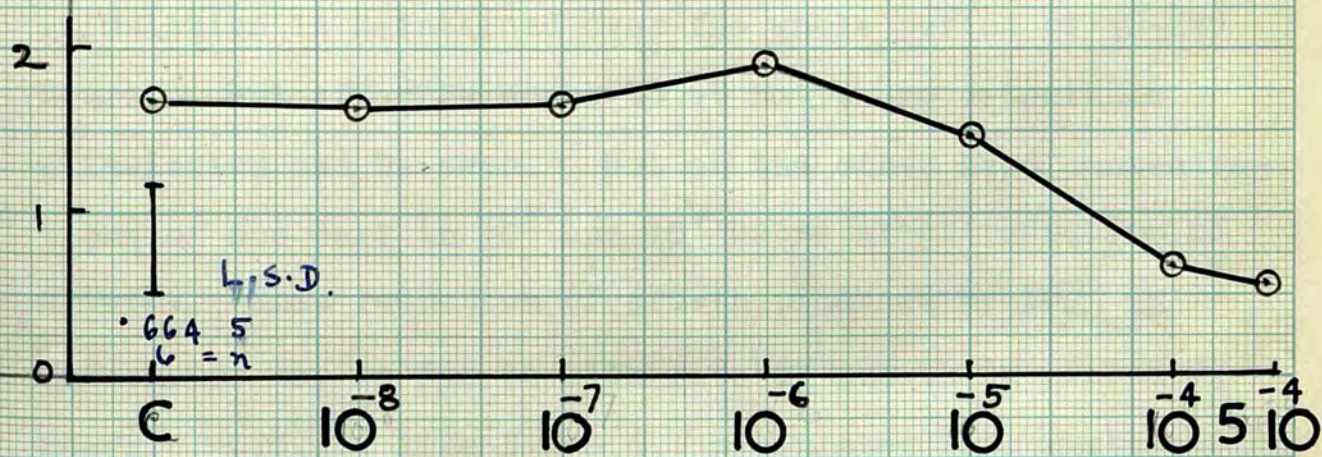
FIG 20 B



7-24 HOURS



24-48 HOURS



CNB CONC GM/ML  
FIG 20C



The action curves with time were also determined in the usual way by calculating the rate of elongation over the period of (0-4), (4-7), (7-24) and (24-48) hours. They are shown in (Fig. 20, B and C).

Concentration-range curve for the action of C.N.B. with time.

Table 43

Sources of variance	Sum of squares	Degree of freedom	Mean sq. variance	P.
C.N.B. (B)	48.7	6	8.116	Highly significant
Time (T)	1085.6	3	361.66	" "
B X T	103.9	18	5.772	Highly significant
Residual	46.3	140	.3307	

L.S.D.  $n = 6 = \underline{\underline{.664}}$

C.N.B. action curve with time shows that there is a well-marked stimulation even in first four hours. This stimulation is maintained over the whole period of (0-24) hours. But after 24 hours to 48 hours, no detectable stimulation can be seen. But on the other hand concentrations of one part in  $10^5$  and  $10^4$  cause great reduction of growth. This reduction of growth produced by  $10^{-5}$  <sub>gm/ml</sub> in late hours may be due to high rate of growth in preceding hours. C.N.B., like N.M.S.A., penetrates into root sections

Figure 21.

The graph showing the concentration-response curve for the action of 4-fluoro, 3-nitrobenzoic acid (F.N.B.A.) in the extension growth of root sections over 48 hours. The vertical line shows the least significant difference at 5% probability level between any two means. The figure below the line records the number of replicate samples from which each mean of the growth rate was calculated.







very quickly causing immediate response in early hours.

(VI) The Concentration-response curve of F.N.B.A.  
F.N.B.A.-(4-fluoro-3,nitro-benzoic acid)

It is a homologue of 4-chloro-3nitro-benzoic acid (C.N.B.). Minarik et al (1952) reported stimulation by this compound as large as 80% over the control. In the hope of obtaining a larger stimulation than in C.N.B. this compound was used.

As before, various concentrations from one part in  $10^8$  to one part in  $10^4$  were used. The plan of the experiments is the same as for C.N.B. The action curve was determined at 48 hours. A slight stimulation about 8% was obtained with concentration one part in  $10^5$ . This low stimulation is probably due to some impurities in the sample which modify the rate of entry of F.N.B.A. into the plant cells. The action curve is shown in (Fig. 21.)

Discussion:

A comparative survey of the action curves of all the compounds shows that there is a marked difference in growth response with the particular phase of the section growth. In spite of this difference in behaviour in relation to the phase of extension growth, the broad nature of the growth

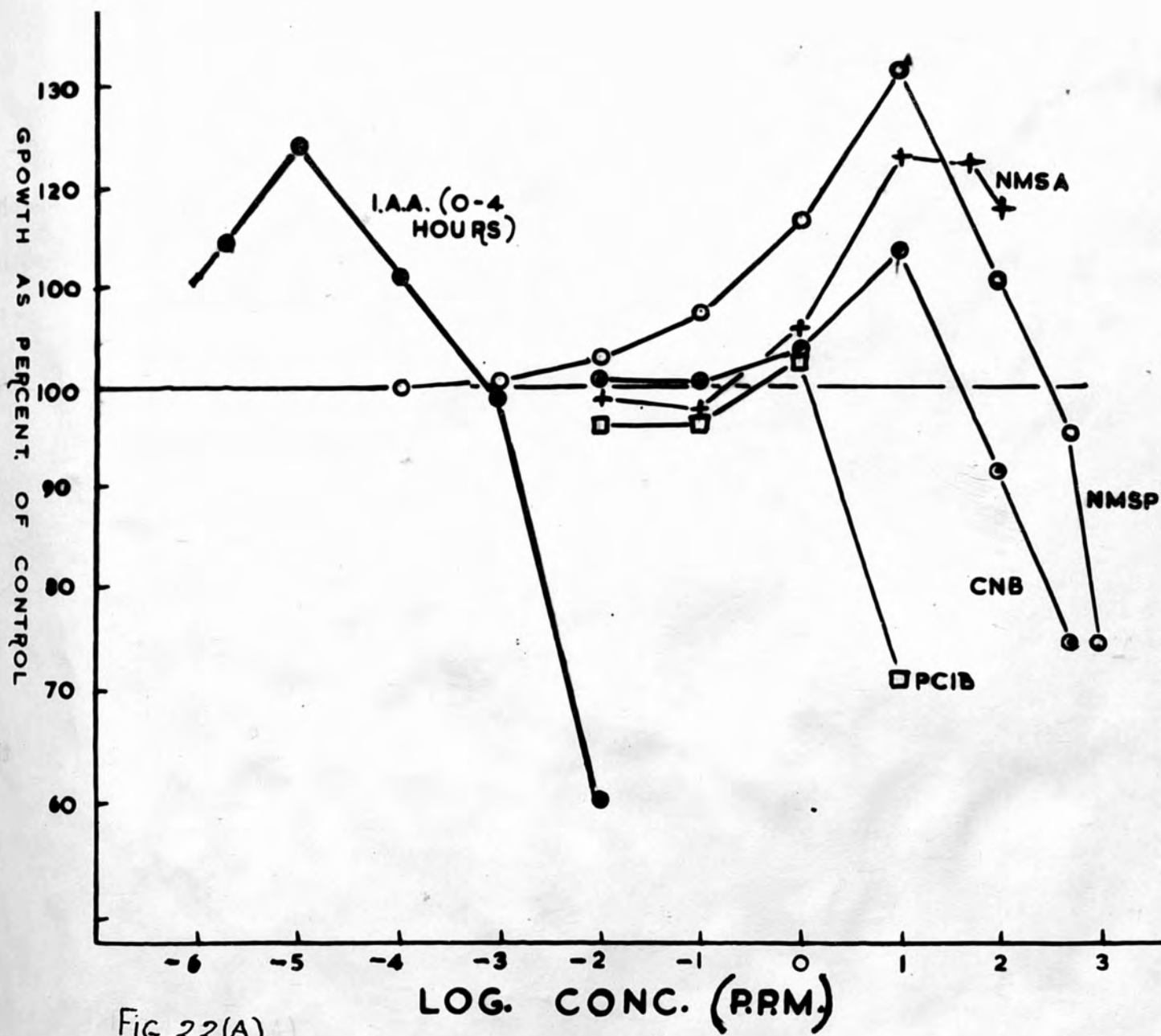


### Figure 22

Concentration-response curves for the action of B-indolyl-acetic acid (IAA) and four antiauxins (N.M.S.P. =  $\alpha$ -(1-naphthylmethyl-sulphide)-propionic acid, N.M.S.A. = 1-naphthyl-methyl-sulphide-acetic acid, C.N.B. = 4-chloro-3-nitro-benzoic acid and P.C.I.B. = p-chloro-phenoxy-iso-butyric acid) on the growth of excised pea root sections.

- A) Plotted strictly against log concentration in parts per million.
- B) Curves shifted without further alteration along the concentration axis until their sub-optimal rising portion coincided.

The antiauxin curves are from over-all extension in the first 24 hours. The IAA curve is for the maximal growth response of the first 4 hours. (Fig. 22 A and B are due to Professor L.J. Audus).





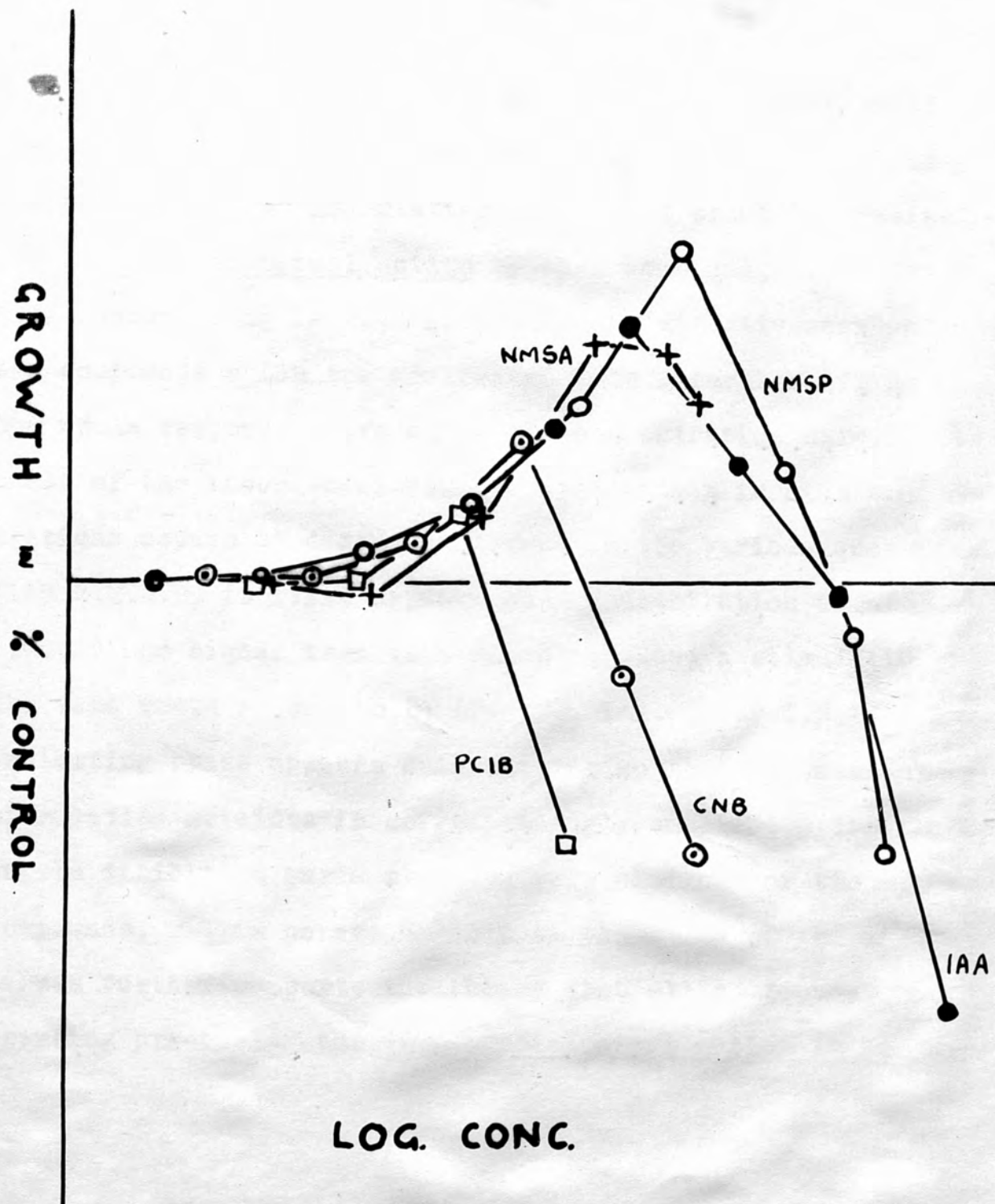


FIG 22 (B)

response is markedly similar for those compounds (Fig. 22A).

The shapes of the ascending limbs of the stimulation curves for IAA, N.M.S.P., C.N.B., and N.M.S.A. <sup>and P.C.I.B.,</sup> are very alike.

This is clearly shown in (Fig. 22 B), where the curves of the optimum response have been superimposed. This clearly suggests that such stimulation is brought about by precisely the same physiological action by each compound, the only differences being in the "activity" or "effectiveness" of the compounds which are manifested by a lateral shifting of the whole response curve along the concentration axis. The onset of the independent "inhibiting" action in high concentrations occurs at different levels in the various compounds. With N.M.S.P. it first appears at a concentration about 1,000 times higher than that which produced a stimulation. The same would appear to be true for IAA. For C.N.B. the inhibiting phase appears much earlier so that the maximum stimulation obtained is correspondingly smaller. The shapes of the inhibition curve are also very similar for these compounds. This correspondence in the shape of the action curves further supports the theory that all compounds are exerting precisely the same physiological action in roots.



## 5. The Interaction of Antiauxins with IAA

### (I) (a) Studies on the interaction of N.M.S.P. with IAA

Two concentrations of IAA (i.e. one part in  $10^{11}$  and one part in  $10^{10}$ , (both concentrations are stimulatory) were used in combination with a range of concentrations of N.M.S.P. from one part in  $10^8$  to one part in  $10^5$ .

From the action curve of N.M.S.P. (See Fig. 17) it is seen that a wide range of concentrations of N.M.S.P. stimulate the growth of root sections. It was therefore considered necessary to use a range of concentration of N.M.S.P. from ( $10^{-8}$  -  $10^{-5}$ )  $\text{gm/ml}$ . Experiments were performed on a strictly orthogonal basis so that full statistical analysis of variance could be performed upon them. The major interaction experiment was broken down into a series of smaller experiments in which the two IAA concentrations were studied. The typical plan for one experiment is given here:

Table 44

### Interaction of N.M.S.P. with IAA

#### Plan of individual experiment

Conc. of N.M.S.P. (gm/ml.)	Conc. (gm/ml.) IAA		
	0	$10^{-11}$	$10^{-10}$
0	2	2	2
$\times$ any one conc.	2	2	2

For each concentration of N.M.S.P., three such experiments were performed to ensure a high degree of precision in the estimation of residual error.

The growth was estimated at intervals of (0-4), (4-7), (7-24) and (24-48) hours, by measuring the fresh weight of sections with a micro torsion balance to the nearest 0.1 mg.

The plan of the full investigation is shown below:

Table 45

N.M.S.P.-IAA Interaction

The plan of full investigation.

conc. (gm/ml.) IAA	Conc. (gm/ml.) N.M.S.P.				
	0	$10^{-8}$	$10^{-7}$	$10^{-6}$	$10^{-5}$
0	24	6	6	6	6
$10^{-11}$	24	6	6	6	6
$10^{-10}$	24	6	6	6	6

The rate of extension per hour was calculated as a percentage of the initial size of sections for each interval. The interaction curves were plotted against the concentrations of N.M.S.P. for each of the concentrations of IAA. In this way four sets of <sup>three</sup> ~~tree~~ graphs were drawn. These are presented in (Fig. 23 A, B, C, D).

The rates of elongation were accumulated in one big table, shown in Appendix. An analysis of variance was



Figure 23

Graphs showing the interactions between B-indolyl-acetic acid (IAA) and a-(1-naphthyl-methyl-sulphide)-propionic acid (N.M.S.P.) in the stimulation of extension growth of excised/<sup>pea</sup> root sections. The times noted above are the growth periods after excising from the parent root. The vertical lines represent the least significant differences at the 5% probability level between the means of growth rates in the series of concentration combinations against which they are placed. The figures against these lines record the numbers of replicate samples from which each mean of the series was calculated.



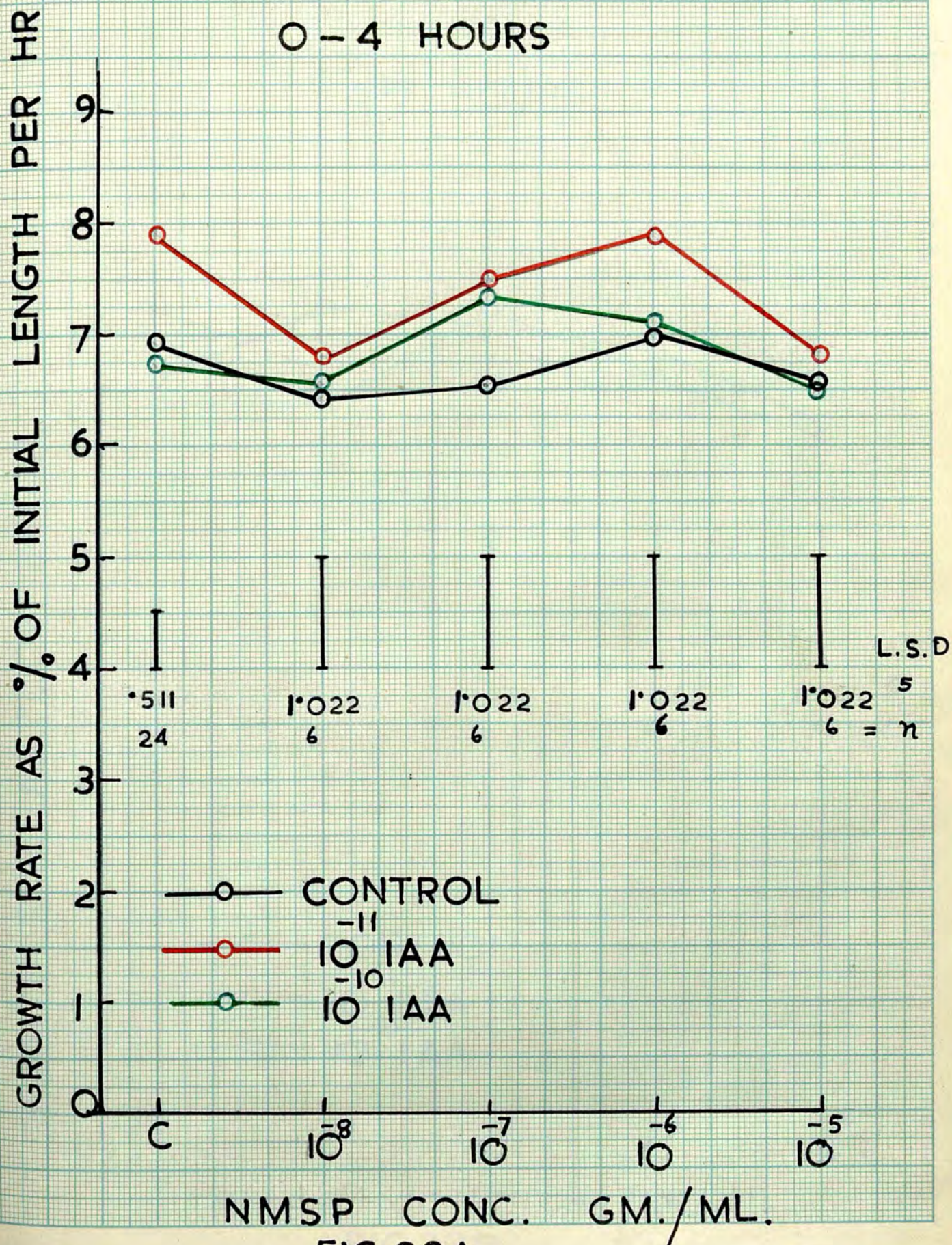


FIG 23A



GROWTH RATE AS % OF INITIAL LENGTH PER HR

4-7 HOURS

9  
8  
7  
6  
5  
4  
3  
2  
1  
0

C

$10^{-8}$

$10^{-7}$

$10^{-6}$

$10^{-5}$

—○— CONTROL  
—○—  $10^{-11}$  IAA  
—○—  $10^{-10}$  IAA

•633  
24

1•266  
6

1•266  
6

1•266  
6

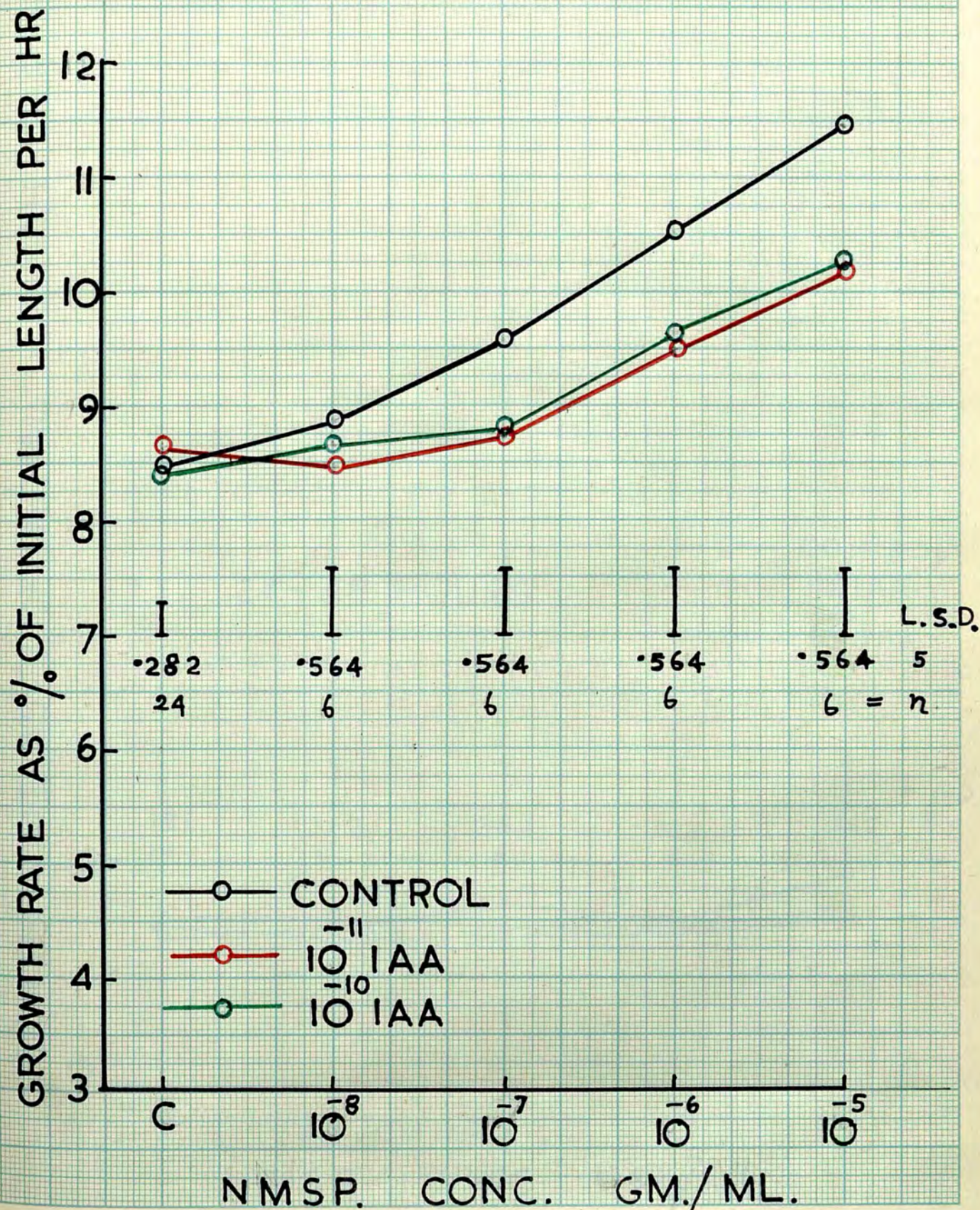
1•266 5  
6 = n

L.S.D

NMSP. CONC. GM./ML.  
FIG 23B



7-24 HOURS





GROWTH RATE AS % OF INITIAL LENGTH PER HR

24 - 48 HOURS

—○— CONTROL  
—○—  $10^{-11}$  IAA  
—○—  $10^{-10}$  IAA

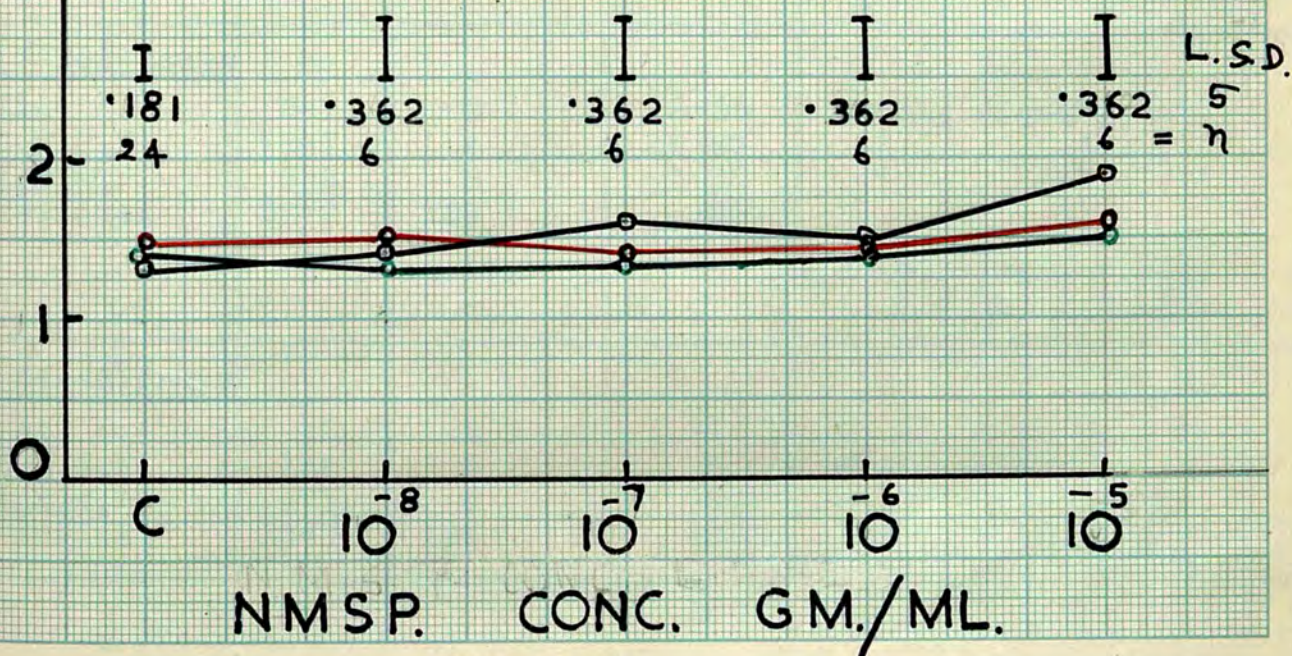


FIG 23D



performed upon the whole results. The results of this are shown below:

Table 46

N.M.S.P.-IAA Interaction in the  
extension growth of pea root sections

Sources of variance	Sum of squares	Degrees of freedom	Mean sq. variance	P.
<u>Time period (0-4) hours</u>				
IAA(I)	20.5	2	10.25	highly significant. at 5% level.
N.M.S.P.(N)	9.5	4	2.375	
Interaction I.X.N.	5.8	8	.725	-
Residual	101.3	129	.785	n = 24, L.S.D. 5% = .511 6 " = 1.022
<u>(4-7) hours</u>				
IAA	6.6	2	3.3	5-6% level.
N.M.S.P.	5.7	4	1.42	
I.X.N.	9.1	8	1.137	-
Residual	155.1	129	1.202	n = 24, L.S.D. 5% = .633 6 " = 1.266
<u>(7-24) hours</u>				
IAA	5.1	2	2.55	highly significant.
N.M.S.P.	78.7	4	19.67	
I.X.N.	9.2	8	1.15	" "
Residual	30.9	129	.239	n = 24, L.S.D. = .282 6 " = .564
<u>(24-48) hours</u>				
IAA	.2	2	.1	- at 5% level.
N.M.S.P.	1.2	4	.3	
I.X.N.	1.1	8	.137	-
Residual	12.7	129	.098	n = 24, L.S.D. = .181 6 " = .362



### Results:

The above variance table shows that the effect of IAA is highly significant at (0-4) hour interval, but this effect of IAA is only significant at 5-6% level at the interval of (4-7) hours. The NMSP effect just reaches significance in the first 4 hours. This is due to low growth values in  $10^{-8}$  gm/ml and  $10^{-5}$  gm/ml and may be inter-experimental variation since NMSP and experiment effects were unavoidably confounded. This is borne out by the lack of any significant NMSP effect in the 4-7 hour period. In the next period (7-24 hours) the NMSP stimulation is clear and highly significant.

The interaction between IAA and NMSP is not significant at first two periods, i.e. (0-4), (4-7) hours, but it is highly significant at (7-24) hours. There is no interaction in the last phase of growth of sections, i.e. in (24-48) hours.

### Discussion:

A careful inspection of the graphs (Fig. 23 A,B) shows that there is no consistent effect of any concentration of NMSP in first seven hours of growth. This may be due to delay in penetration of NMSP into root cells. The full effect which is highly significant becomes apparent only in the (7-24) hour period. This is seen in (Fig. 23 c). In contrast to this NMSP effect, the maximum IAA stimulation in  $10^{-11}$  ~~gm/ml~~ was immediate in first four hours. In (4-7) hour period, there is a smaller but still significant effect of IAA and then it virtually disappears after (24-48) hours.

Analysis of the results shows that in the first two growth periods there is no significant interaction between these two substances, which may be interpreted to mean that in this period N.M.S.P. may not have penetrated in sufficient quantities into the cell to exert an effect on growth centres. In (7-24) hour, the interaction variance is highly significant. The graphs in (Fig. 23 C) show that this is due to a marked reduction in the stimulatory effects of N.M.S.P. by both concentrations of IAA, in spite of the fact that IAA at this time has no effect on the growth rate. In (24-48) hour interval there is also no interaction between them.

Three explanations may be put forward to account for the latter effect.

Firstly, it might be suggested that IAA, after penetrating into the cell and there evoking the growth response, is converted into an inactive (or perhaps even inhibitory) derivative which itself antagonises the N.M.S.P. effect. This would agree well with the recent suggestions by Bennet-Clark and Kefford (1954) who interpreted their growth rate time curves for coleoptile sections in high auxin concentrations along the same lines. Secondly, it is possible that decreased growth rate in this period (7-24), (24-48) hours, may result from the higher growth rate in the first two periods. If optimal total extension is in fact limited,



under the action of both types of compounds, by other unknown factors, then a stimulation in early phase will necessarily mean a slower rate of growth in a later phase. From many points of view this seems unlikely. The third possibility is that IAA in the external solution, may impede the entry of N.M.S.P. still further, thus reducing the degree of stimulation in this phase.

(b) Sections pretreated with N.M.S.P., washed and treated with IAA.

In order to test the last alternative mentioned above, a slightly modified experiment was conducted, in which a series of samples were pretreated with N.M.S.P. at a concentration of one part in  $10^5$  for a period of six hours after excision. This was done in order to ensure the penetration of N.M.S.P. into the growing cells. A Similar series were grown as controls in  $\frac{1}{2}\%$  sucrose solution alone. At the end of first six hours the extension growth of the sections was measured and from these data, the rate of extension growth in N.M.S.P. and control samples was calculated. The graph is shown in (Fig. 24)A

An analysis of variance was performed on the data to see if the effect of N.M.S.P. treatment for the first six hours is significant or not. The analysis of variance is shown in the table on the next page:

Figure 24

Results of N.M.S.P. pretreatment at a concentration of  $10^{-5}$  gm/ml.

- A. Growth during pretreatment.
- B. Growth subsequent to removal of N.M.S.P. and under the action of various concentrations of 1AA.

The vertical lines show the least significant differences between means of growth rates at the 5% probability level. The figures below the lines record the number of replicate samples from which each mean was calculated.



GROWTH RATE AS % OF INITIAL LENGTH PER HR

0-6 HOURS

I  
•316  
24

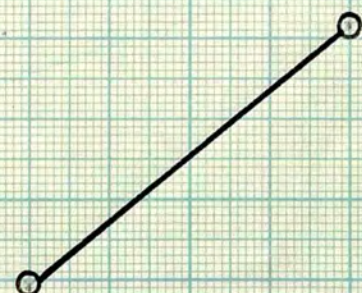
I  
•316  
24

C

$10^{-5}$

NMSP. CONC. GM./ML.

FIG 24 A





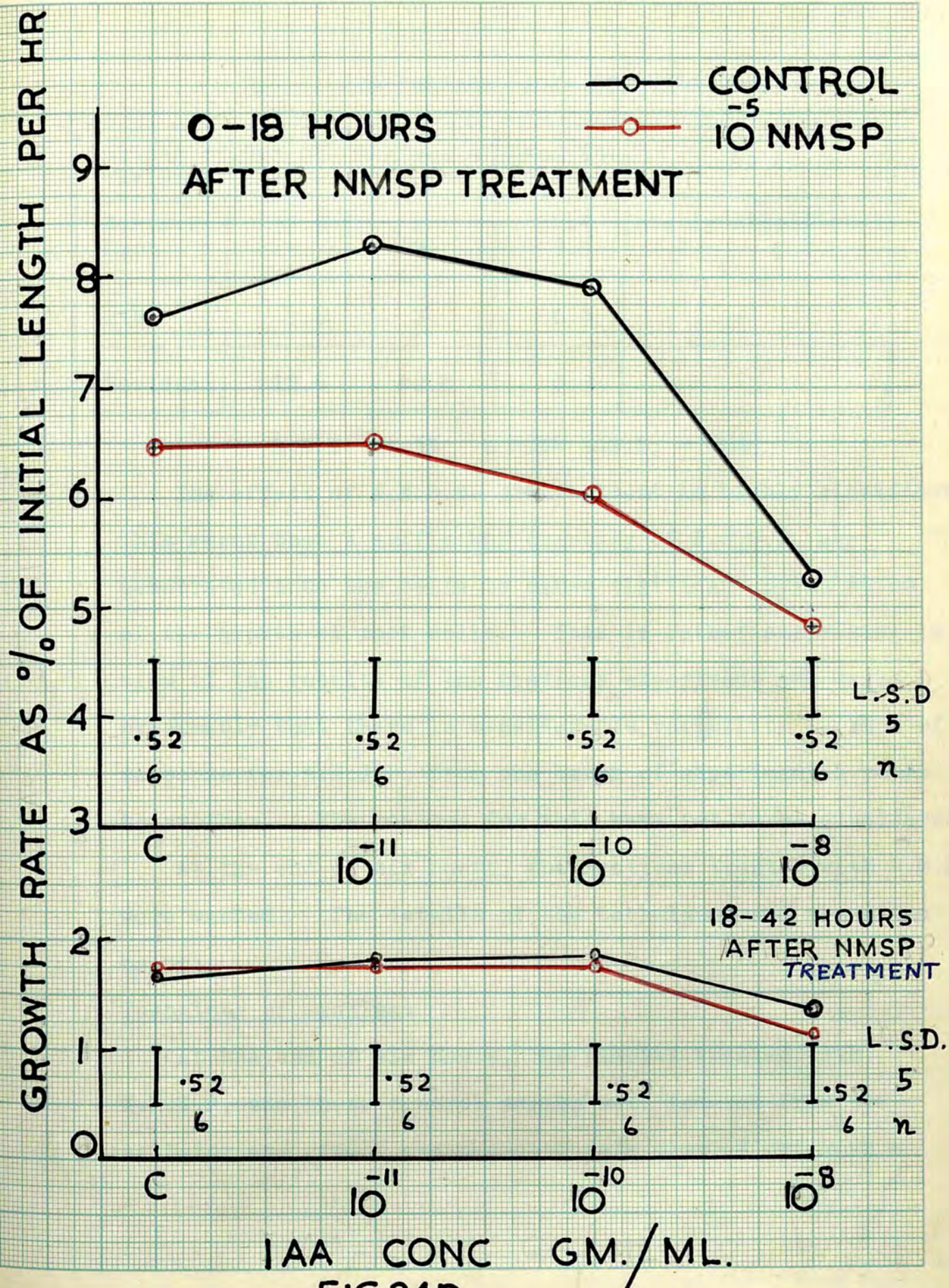


FIG 24B



Table 47

N.M.S.P. Pretreatment for first six hours

Sources of variance	Sum of squares	Degrees of freedom	Mean sq. variance	P.
N.M.S.P. Expt.	32.9 19	1 2	32.9 9.5	highly significant "
EXN	1.6	2	0.8	-
Residual	12.6	42.0	0.3	

L.S.D. at 5%  $n = 24$  L.S.D. = '316

The effect of N.M.S.P. pretreatment is highly significant. This means that a sufficient quantity of N.M.S.P. has penetrated into the cells to cause significant stimulation of the growth of root cells. This is rather surprising in view of the results of the previous experiment and means presumably that there is considerable variability in rate of response for N.M.S.P. from one batch of experimental roots to another. The reasons for this have yet to be elucidated.

At the end of six hours, the sections treated with N.M.S.P. were washed. The details of the technique used are given below:

Technique of washing:

At the end of six hours, the samples treated with N.M.S.P. and those control series were removed from the agitator and growth measurements were carried out with a

micro-torson balance. The root sections, treated with N.M.S.P., were then washed in a Buchner funnel with  $\frac{1}{2}\%$  sucrose solution. Each sample of 10 sections was separately washed for about 8 minutes and then transferred to fresh, clean, sterile petri-dishes.



The washed sections were then treated with three concentrations of IAA, two stimulatory (one part in  $10^{11}$  and  $10^{10}$ ) and one inhibitory (one part in  $10^8$ ). Control samples in  $\frac{1}{2}\%$  sucrose solution were simultaneously observed. The sections were again weighed at 18 and 42 hours subsequent to the pretreatment. The rates of growth per hour were calculated as a percentage of the initial size of sections. The results are reproduced in (Fig. 24 B<sub>4</sub>).

The results were then subjected to analysis of variance and the table is given below:

Table 48

Interaction of IAA and N.M.S.P. (washed off)  
after 6 hours

Sources of variance	Sum of squares	Degrees of freedom	Mean sq. variance	P.
IAA(I)	26.4	3	8.8	highly significant.
N.M.S.P.(N)	9	1	9.0	1%
Time (T)	582.6	1	582.6	highly significant.
IXN	2.9	3	.96	1% level.
NXT	12.6	1	12.6	highly significant.
IXT	15.4	3	5.1	" "
IXNXT	1.5	3	.5	5% level.
Residual	15.9	80	.199	

at 5% level

L.S.D. =  $\pm$

for  $n = 6$ , L.S.D. = .52

### Results:

Over the 18 hour period subsequent to pretreatment, sections pretreated with  $\frac{1}{2}\%$  sucrose alone gave normal growth responses to IAA. Those samples pretreated with N.M.S.P., however, all showed a much smaller growth than the controls after washing and in addition IAA at both concentration  $10^{-11}$  and  $10^{-10}$  gm/ml gave no growth stimulation. This interaction of N.M.S.P. and IAA is significant at 1% level. This is shown in the variance table presented above. Inhibition by one part in  $10^8$  IAA was also much smaller than in controls, but was still significant. This "residual" effect of N.M.S.P. is very puzzling. It cannot be interpreted in terms of the limitation of total extension by other unknown factors. (See second alternative p.88) since (a) growth continues at high rate if N.M.S.P. is not removed by washing and (b) the small extra extension during the pretreatment period is far less than the subsequent reduction of extension after washing. This experiment shows conclusively that N.M.S.P. which had entered the root antagonised both the stimulation and inhibition of growth by IAA applied later.

(c) Sections pretreated with IAA, washed, treated with N.M.S.P.

This experiment was performed in order to check whether N.M.S.P. antagonism might be a prevention of IAA entry into the root sections.



Figure 25.

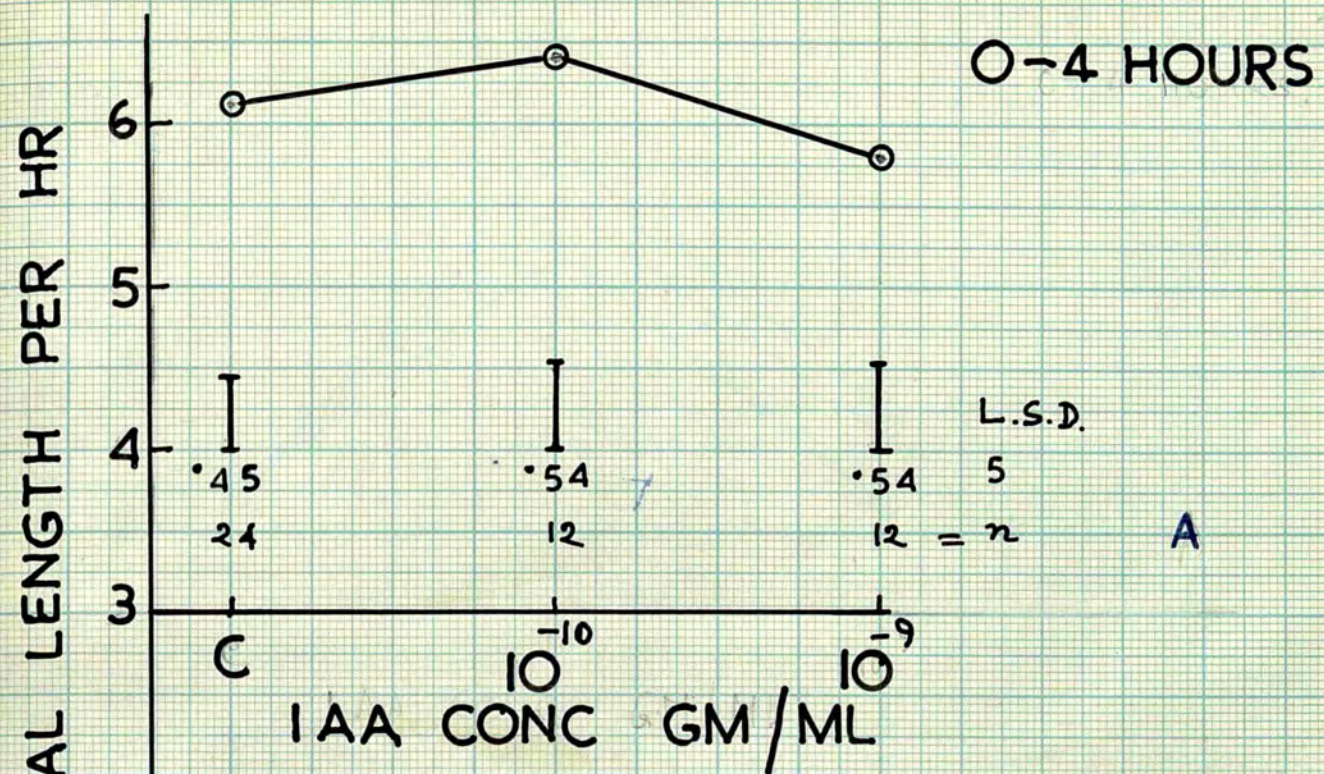
Effects of pretreatment with 1AA at concentrations of  $10^{-10}$  and  $10^{-9}$  gm/ml.

A. Growth during pretreatment.

B, C Growth after removal of 1AA under the action  
& D of  $10^{-5}$  and  $10^{-4}$  gm/ml N.M.S.P.

The vertical lines show the least significant differences between means of growth rates at the 5% probability level. The figures against these lines record the numbers of replicate samples from which each mean of the series was calculated.





**0-3 HOURS  
AFTER IAA  
TREATMENT**

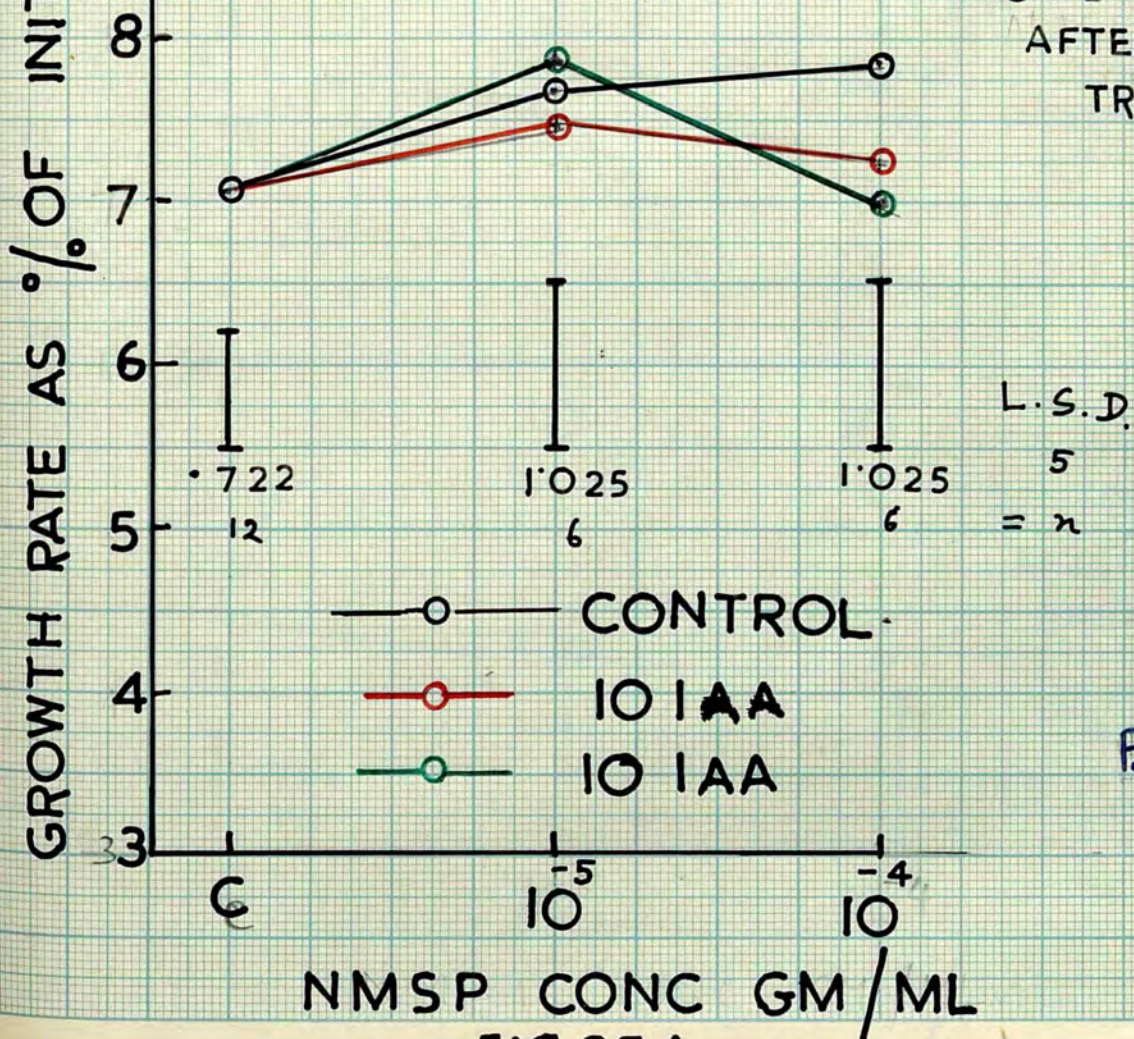
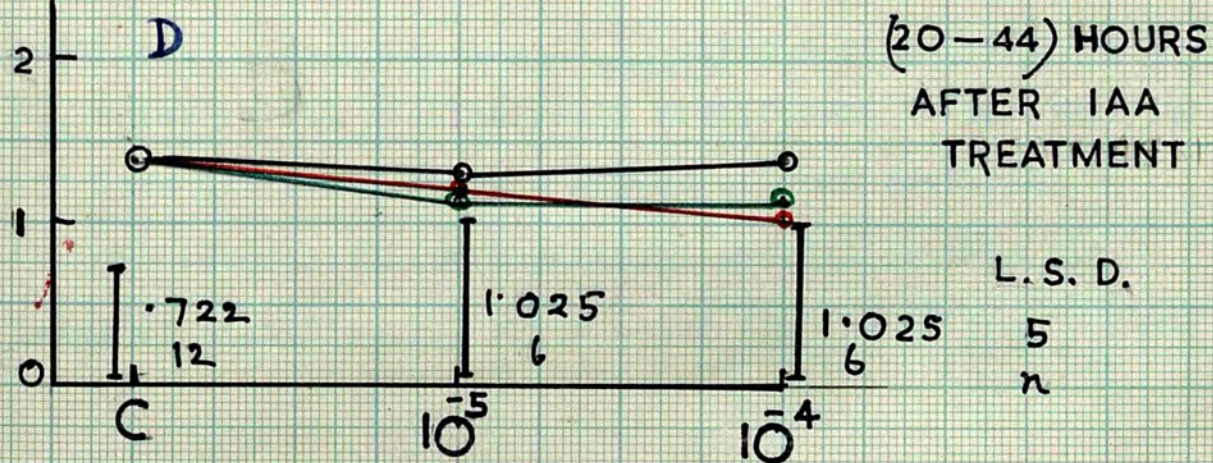
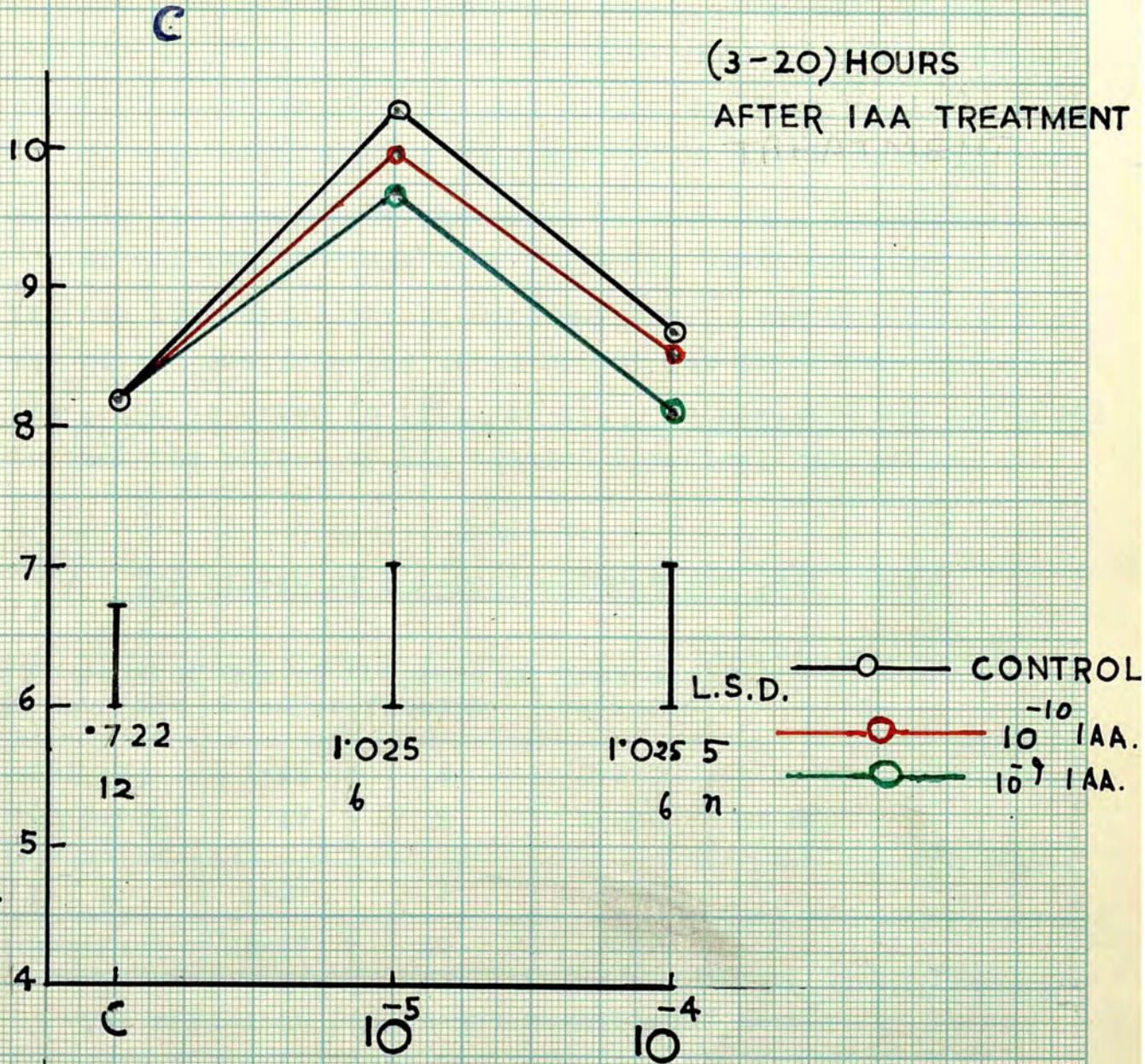


FIG 25 A



GROWTH RATE AS % OF INITIAL LENGTH PER HR



NMSP CONC GM./ML.  
FIG 25 B



Two concentrations (one part in  $10^{10}$  and  $10^9$ ) of IAA were used. These two concentrations would be at the highest possible concentration without giving an inhibition.

In this experiment a procedure was adopted which was exactly the reverse of that used in preceding experiment.

At the end of first four hours the sections treated with IAA and also the controls were weighed. The rate of growth was worked out from the total growth for first four hours. The graph is shown in (Fig. 25 A). An analysis was performed to ~~list~~<sup>test</sup> the effect of IAA treated for first four hours:

Table 49

The effect of IAA-pretreatment for (0-4) hours

Sources of variance	Sum of squares	Degrees of freedom	Mean sq. variance	P.
IAA (I)	1.99	2	0.995	at 5%
EXPT (E)	4.47	2	2.235	
I X E	1.57	4	.392	
Residual	24.67	39	.632	

L.S.D at 5%,  $n=24$  L.S.D = .45;  $n=12$ , L.S.D = .54

The effect of IAA is not significant because at these concentrations IAA does not stimulate the growth of root sections. Concentration  $10^{-10}$  <sup>gm/ml</sup> gives a stimulation of about 5%, which is not statistically significant. One part in  $10^9$  does not stimulate the growth of root sections.

The interaction of experiment and IAA reaches the level of significance, which means that IAA effect varies



from experiment to experiment.

At the end of first four hours IAA-treated sections were then washed in the manner described in the previous experiment. The washed sections were treated with N.M.S.P. (one part in  $10^5$  and  $10^4$ ). They were allowed to grow for a period of 44 hours subsequent to pretreatment of IAA.

At the interval of (0-3), (3-20), (20-44) hours subsequent to the pretreatment of IAA, sections were weighed. The graphs shown in (Fig. 25 B, C, D) were drawn from the rate of growth per hour.

The results were subjected to analysis of variance which are shown below:

Table 50

IAA-N.M.S.P. Interaction subsequent to pretreatment of IAA

Sources of variance	Sum of squares	Degrees of freedom	Mean sq. variance	P.
IAA(I)	.1	1	.1	-
N.M.S.P.(N)	16.3	2	8.15	0.1% level.
Time (T)	1586.4	2	793.2	highly significant.
IXN	3	2	1.5	at 15% level.
NXT	17.1	4	4.27	at 1% level.
IXT	1.1	2	0.55	-
IXNXT	1.3	4	.32	
Residual	99.9	126	.79	

L.S.D. at 5%;  $n = 12$   
 $n = 6$

L.S.D. = .722  
 " = 1.025

Discussion:

The analysis shows that there is a highly significant N.M.S.P. effect and a highly significant interaction with time. The graphs show that this is accounted for by a slight non-significant stimulation by N.M.S.P. 0-3 hours after IAA pretreatment and a large highly significant stimulation in the 3-20 hour interval. Between 20 and 44 hours all effects disappear. There is no significant residual effect of IAA pretreatment but the IAA x N.M.S.P. interaction variance is large enough to reach the 15% level of significance. Inspection of the graphs show that this is due to a reduction of the strength of N.M.S.P. stimulation at  $10^{-4}$  gm/ml by both concentrations of IAA in the 0-3 hours and a similar slight reduction in both N.M.S.P. concentrations in 3-20 hour period. Although this variance is too small to constitute satisfactory proof of an antagonism it suggests that IAA remaining in the section may have antagonised the action of N.M.S.P. entering subsequently and that the antagonism may not therefore be IAA retardation of N.M.S.P. entry. Further critical experiments are needed along these lines.

(d) Studies on the interaction of N.M.S.P. and IAA in light and dark

It was indicated in previous experiment (see Page 32-33) that destruction of IAA may take place in light. This inactivation of IAA in light causes a shift in optimum from one part in  $10^{11}$  to one part in  $10^{10}$ . It can therefore



be expected that in light the degree of antagonism between IAA and N.M.S.P. will be less than in dark.

To test this inactivation of IAA and the consequently reduced antagonism between them, experiments were conducted

simultaneously in dark as well as in light.

Two stimulatory concentrations, one part in  $10^{11}$  and one part in  $10^{10}$  of IAA, were used in combination with a range of concentrations of N.M.S.P. from (one part in  $(10^8 - 10^5)$ ).

The sections to be grown in dark were cut in dim red light and those to be grown in light were cut in strong light of intensity 100 watt.

The plan of the experiment is shown below:

Table 51

IAA-N.M.S.P. Interaction in light and dark

Plan of individual experiment

(Conc.) N.M.S.P.	Dark			Light		
	Conc. (gm/ml.) IAA			Conc. (gm/ml.) IAA		
	0	$10^{-11}$	$10^{-10}$	0	$10^{-11}$	$10^{-10}$
0	2	2	2	2	2	2
(x) any one conc.	2	2	2	2	2	2

The treated and control samples were allowed to grow in light (intensity 120 foot candle) and dark for a period of 48 hours.

Two such experiments were performed for each concentration of N.M.S.P. At the end of 48 hours the sections were weighed. The ratios of the treated samples grown in light and dark were calculated to the corresponding

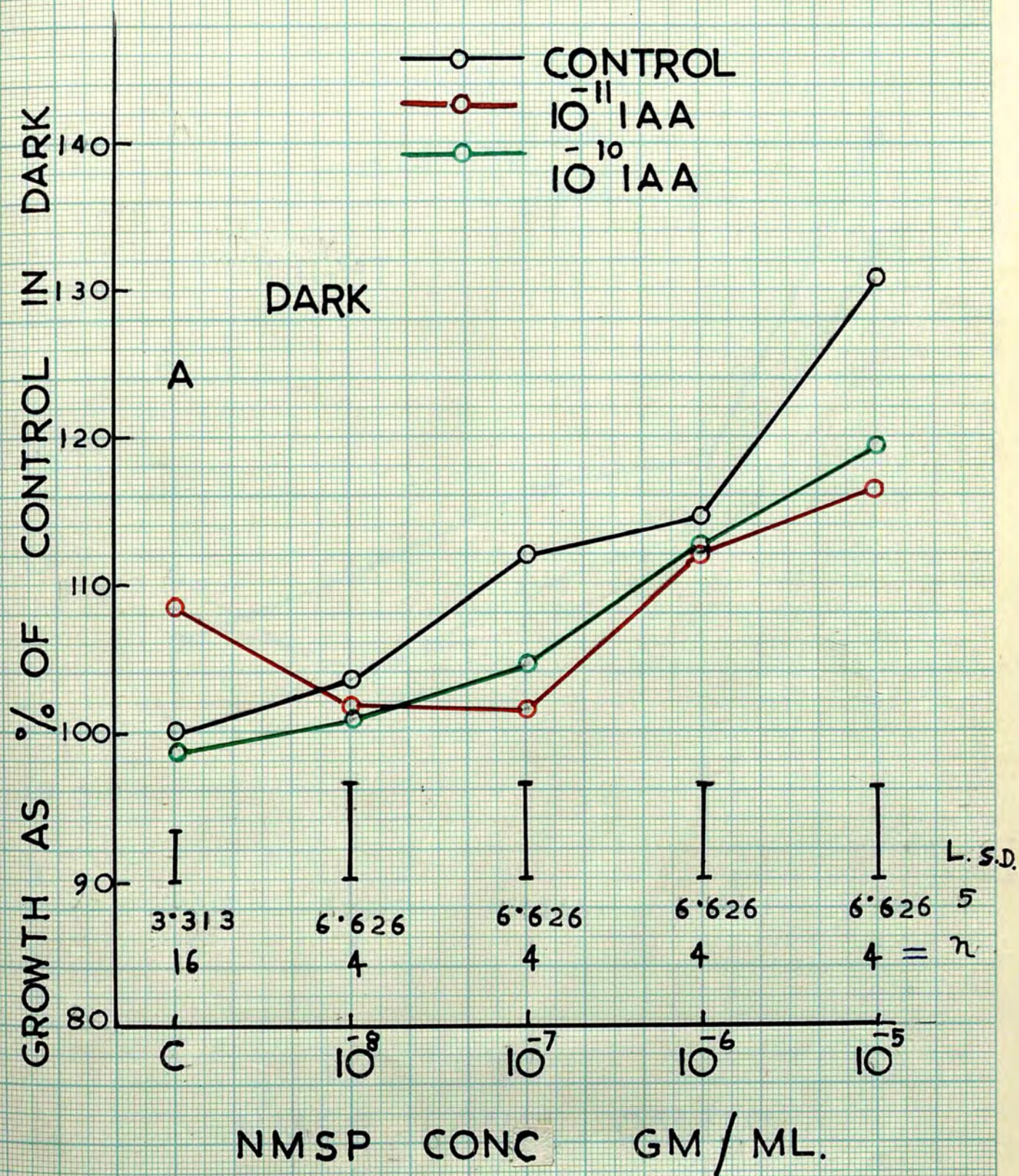


Figure 26

Graphs showing the interactions between B-indolyl-acetic acid (IAA) and a(1-naphthyl-methyl-sulphide)-propionic acid (N.M.S.P.) in light and dark in the stimulation of extension growth of excised pea root sections <sup>over</sup> ~~in~~ 48 hours.

The vertical lines represent the least significant <sup>relevant</sup> differences between/means at the 5% probability level. The figures against those lines record the numbers of replicate samples from which each mean of the series was calculated.







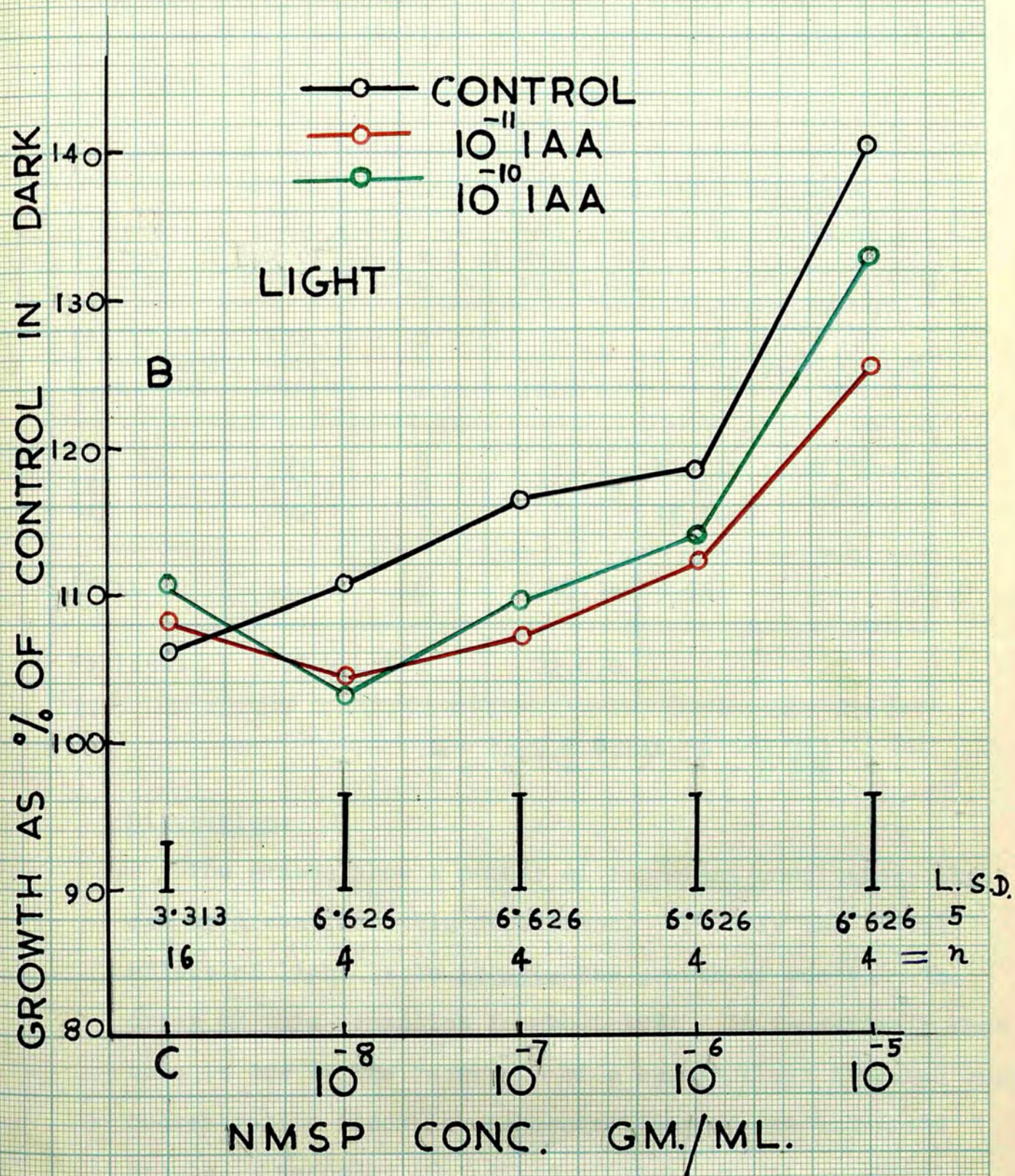


FIG 26B



controls in dark. The results are reproduced in (Fig. 26 A, B). An analysis of variance was performed and is shown below:

Table 52

IAA-N.M.S.P. Interaction in light and dark

Sources of variance	Sum of squares	Degrees of freedom	Mean sq. variance	P.
IAA (I)	172.2	2	86.1	5% level
N.M.S.P.(N)	10686.2	4	2671.5	highly significant
Light (L)	1616.4	1	1616.4	" "
IXN	1899.7	8	237.5	highly significant
IXL	472.1	2	236.05	" "
NXL	292.3	4	73.1	1% level
IXNXL	330.7	8	41.34	5-6%
Residual	3575.4	162	22.070	

$$\begin{aligned} n = 16 \text{ L.S.D. at } 5\% &= 3.313 \\ n = 4 \text{ " " " } &= 6.626 \end{aligned}$$

Discussion:

The graphs show a number of important conclusions that can be drawn from this experiment. Firstly the overall effect of light is to promote the growth of roots. The order of this promotion is about 10%. It is unlikely that this is due to the reduction of a supraoptimal internal auxin concentration since low concentrations of IAA stimulate growth. Secondly the effect of light on IAA stimulation compares with those in the previous experiments since control



results show that in light maximum stimulation is given by  $10^{-10}$  gm/ml and in the dark by  $10^{-11}$  gm/ml IAA. Thirdly the percentage stimulation of growth by N.M.S.P. is virtually the same in both light and dark (about 30%) although the maximum root growth in light under the action of N.M.S.P. is very much increased. This suggests that light does not effect N.M.S.P. action and therefore acts by affecting directly the general activity of the growth system. Lastly there is a marked mutual antagonism of IAA and N.M.S.P. and this seems to be slightly more marked in the light than in the dark (almost significant triple interaction). The reason for this is obscure. If IAA were destroyed by light one might have expected the reverse to <sup>be</sup> the case. Obviously more experiments are needed along these lines.

(II) (a) Studies on the interaction of IAA and P.C.I.B.

It has been seen in previous experiment (p. 79 Fig. 19) (action curve), that P.C.I.B. causes very little stimulation of the growth of the root sections. A very slight stimulation is indicated at a concentration ( $3 \times 10^{-6}$  gm/ml) but this has only just reached the 5% level of significance. This small effect was, however, visible in the first few hours after excision. This compound must therefore enter the sections rapidly.

In the interaction experiments a range of concentrations of one part in ( $10^7 - 10^5$ ) of P.C.I.B. was used in combination with two stimulatory, one part in  $10^{11}$  and  $10^{10}$  of IAA.

Plan of individual experiment is shown below:

Table 53

IAA-P.C.I.B. Interaction

Plan of individual experiment

Conc. of P.C.I.B.	Conc. (gm/ml.) IAA		
	0	$10^{-11}$	$10^{-10}$
0	2	2	2
$\times$ any one concentration	2	2	2

Three such experiments were performed with each of the concentrations of P.C.I.B. used.

The plan of the full investigation is as follows:

Table 54

IAA-P.C.I.B.-interaction

Plan of the full investigation

Conc. (gm/ml.) IAA	Conc. (gm/ml.) P.C.I.B.				
	0	$10^{-7}$	$10^{-6}$	$3 \times 10^{-6}$	$10^{-5}$
0	24	6	6	6	6
$10^{-11}$	24	6	6	6	6
$10^{-10}$	24	6	6	6	6

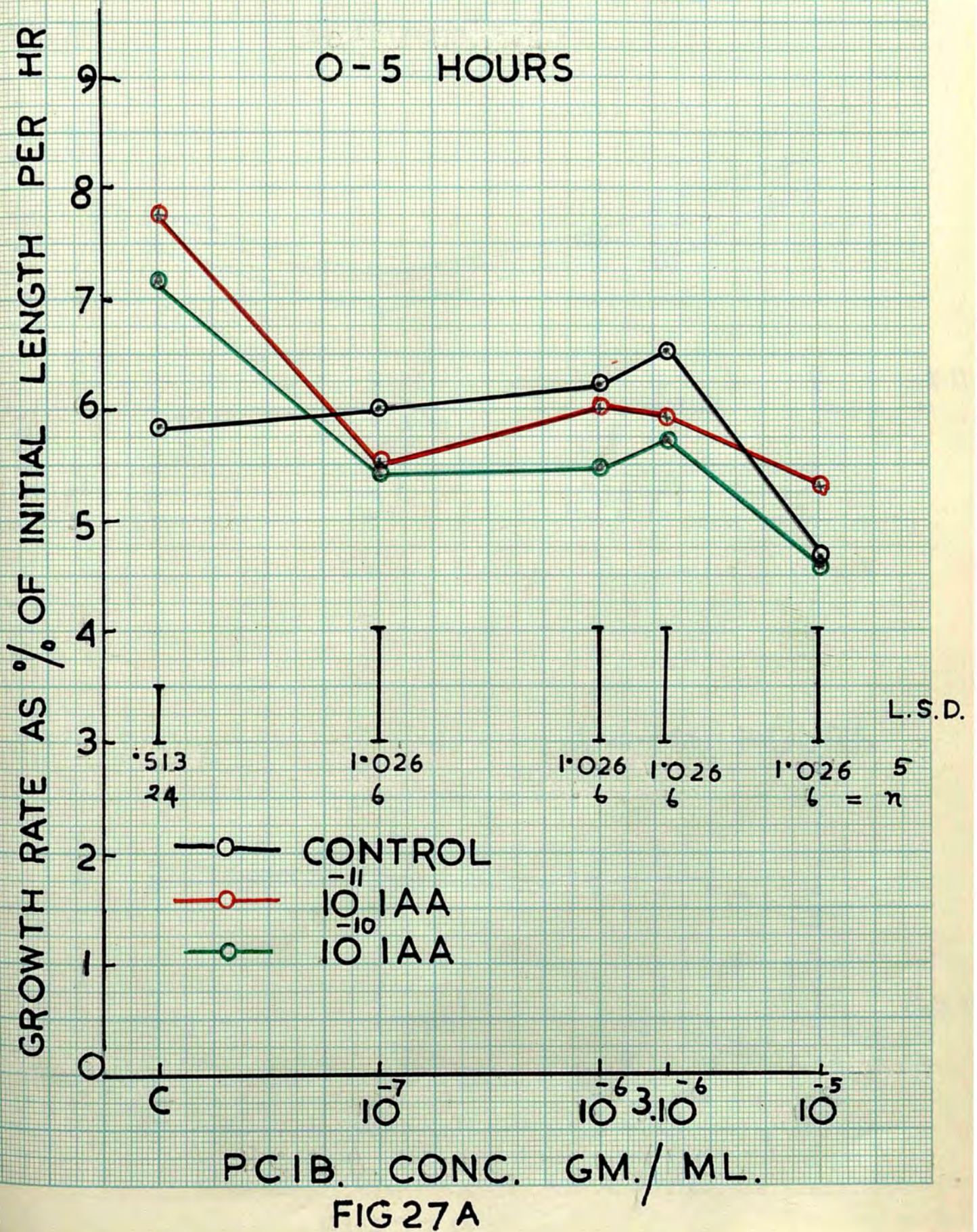
The sections were weighed at the intervals of (0-5), (5-24), (24-48) hours. The rate of growth per hour was calculated from these readings at different intervals.



Figure 27

The graphs showing the interactions between B-indolyl-acetic acid (IAA) and p-chloro-phenoxy-iso-butyric acid (P.C.I.B.) in the stimulation of extension growth of excised pea root sections. The times noted are the growth periods after excision from the parent root. The vertical lines represent the least significant differences at the 5% probability level between the means of growth rates in the series of concentration combinations against which they are placed. The figures against these lines record the numbers of replicate samples from which each mean of the series was calculated.







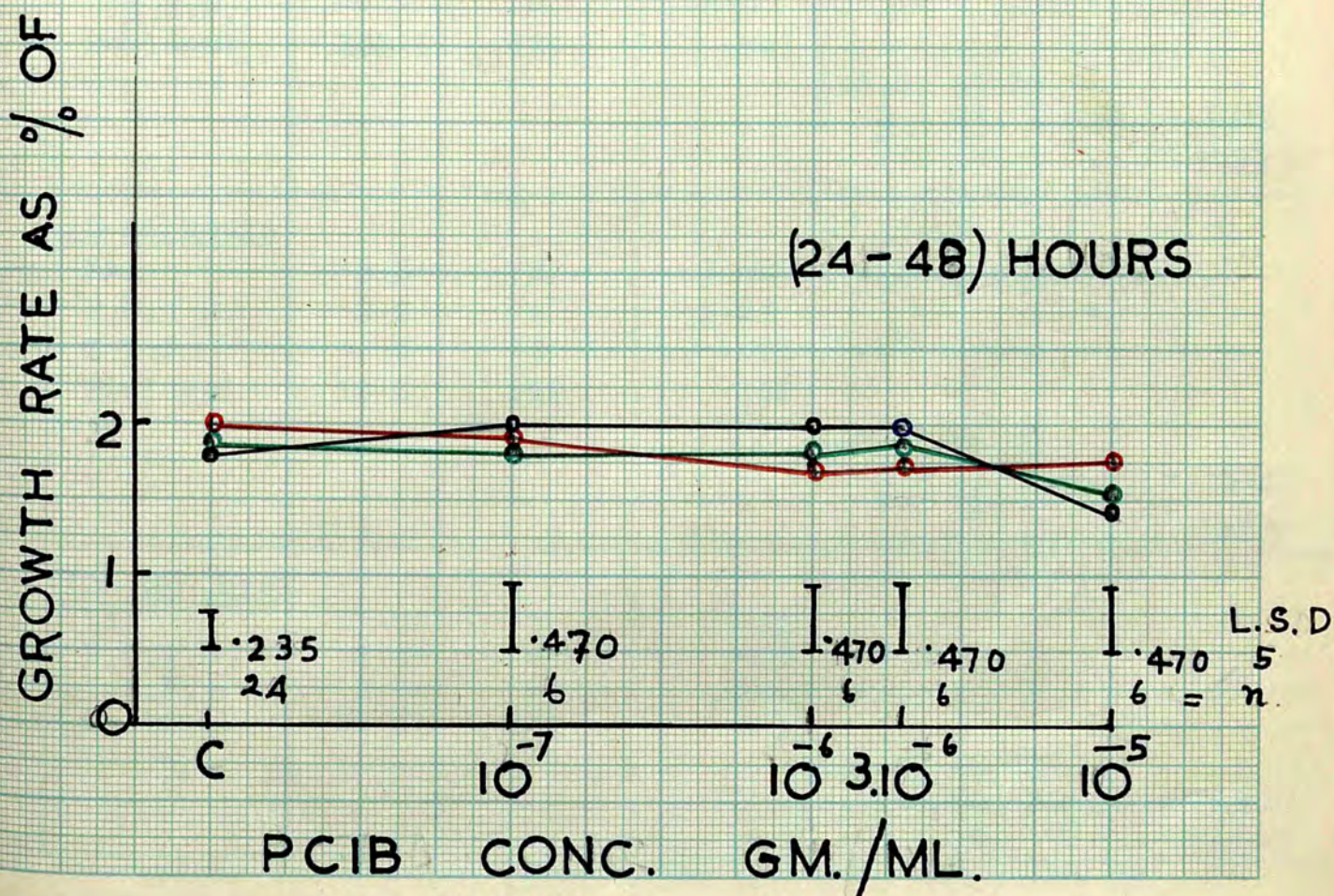
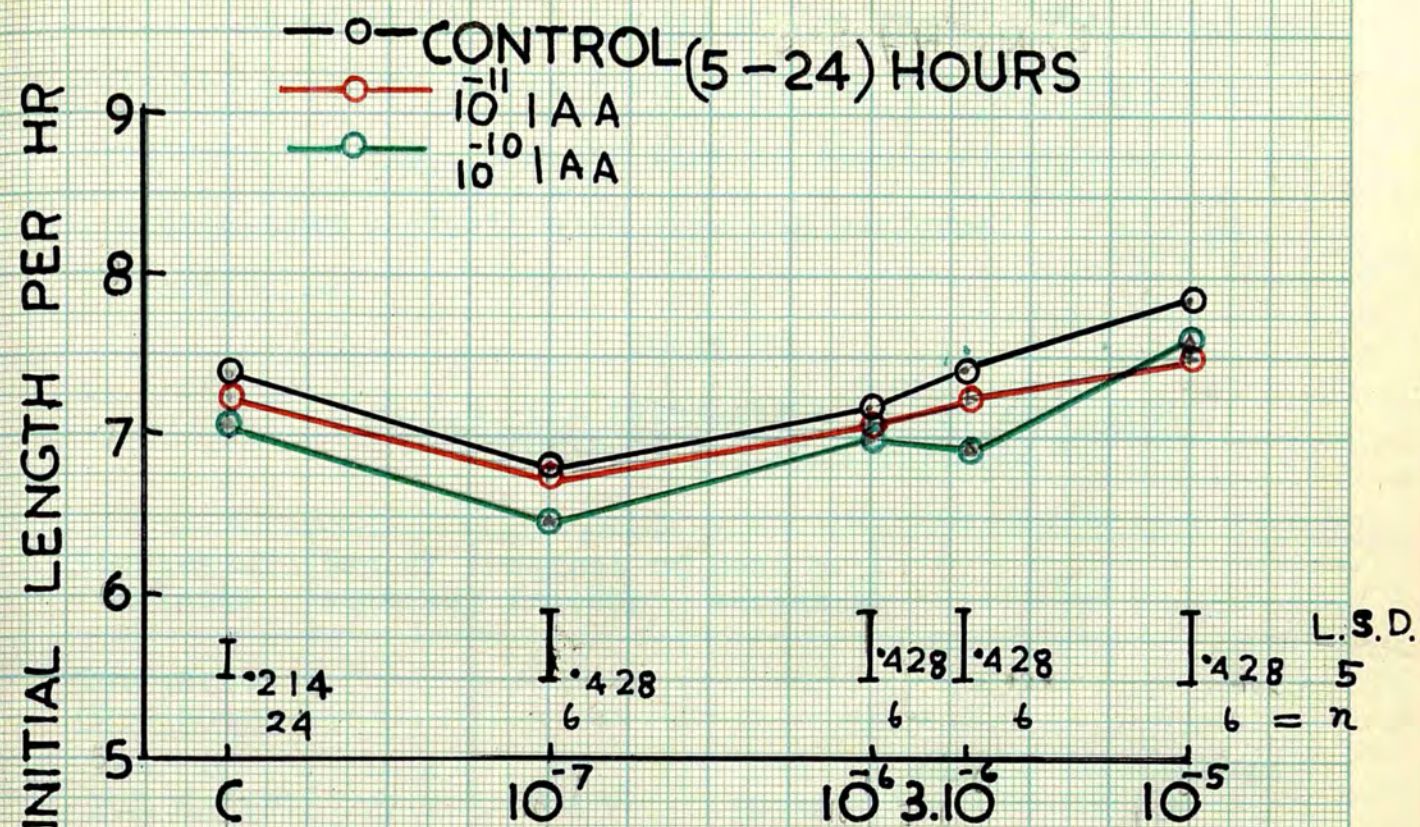


FIG 27 B



An analysis of variance of the results was performed. In the analysis it was found that the triple interaction between P.C.I.B., IAA and time is highly significant. So a breakdown analysis with time was performed. This is shown below:

Table 55

P.C.I.B.-IAA interaction in the extension  
growth of pea root sections

Sources of variance	Sum of squares	Degrees of freedom	Mean sq. variance	P.
<u>Time period (0-5) hours</u>				
IAA I	18.2	2	9.1	highly significant.
P.C.I.B.(P)	77.7	4	19.42	" "
Interaction I.X.P.	36.0	8	4.5	" "
Residual	102.1	129	.791	
				L.S.D. at 5% n = 24, L.S.D. = .513 " 6 " = 1.026
<u>(5-24) hours</u>				
IAA	2.5	2	1.25	highly significant.
P.C.I.B.	8.9	4	2.22	" "
I.X.P.	.6	8	.075	-
Residual	18.8	129	.145	
				L.S.D. at 5% n = 24, L.S.D. = .214 " 6, " = .428
<u>(24-48) hours</u>				
IAA	.05	2	0.025	-
P.C.I.B.	2.42	4	.605	1% level.
I.X.P.	1.04	8	.13	-
Residual	21.37	129	.165	
				L.S.D. at 5% n = 24, L.S.D. = .235 n 6, " = .470

The graphs drawn at different time intervals are shown in (Fig. 27 A, B, &.)



It will be seen that the two concentrations of IAA cause a highly significant stimulation of growth from 0-5 hours but that after this both concentrations give a small inhibition. This second effect has not been observed before. The cause is obscure. The P.C.I.B. effect in the first 5 hours is seen to be a slight stimulation at  $3 \times 10^{-6}$  gm/ml and an inhibition at  $10^{-5}$  gm/ml. In the subsequent period (5-24 hours) the optimum seems to have shifted to  $10^{-5}$  gm/ml for reasons which are not obvious. The highly significant IAA x P.C.I.B. interactions in the first five hours is seen from the graph to be a mutual antagonism such that growth in any mixture is much lower than that in solutions of either of the compounds alone.

(b) Interaction of P.C.I.B. and inhibitory concentration,  $10^{-8}$  of IAA

It has been previously noted that P.C.I.B. has very little stimulatory effect on the growth of root sections. A very slight stimulation is indicated at concentration of  $3 \times 10^{-6}$  gm/ml P.C.I.B., but this scarcely reached the 5% level of significance. Concentrations lower than  $3 \times 10^{-6}$  gm/ml do not stimulate the growth of the sections. The ineffectiveness of

P.C.I.B. in the growth of pea root sections is in strong contrast to the findings of Burström, who obtained about 60% stimulation of wheat roots. It suggests that P.C.I.B. is possibly a weak antiauxin which cannot antagonise the supposed natural growth inhibitor in root. This observation prompted me to do some experiments with various concentrations of P.C.I.B. used in combination with an inhibitory concentration, namely, one part in  $10^8$  of IAA. If P.C.I.B. is not a weak antiauxin then it will effectively alleviate the inhibition caused by IAA. Concentration of P.C.I.B. one part in  $10^5$  was not used, since it is highly inhibitory.

A range of concentrations from one part in  $10^7$  to three parts in  $10^6$  was used in combination with one concentration, i.e. one part in  $10^8$ , of IAA.

The plan of the investigation is given below:

Table 56

P.C.I.B. and IAA Interaction (inhibitory concentration)

Plan of full investigation

conc. IAA (gm/ml.)	Conc. (gm/ml.) P.C.I.B.			
	0	$10^{-7}$	$10^{-6}$	$3 \times 10^{-6}$
0	18	6	6	6
$10^{-8}$	18	6	6	6

The extension growth of the sections was measured under a travelling microscope at intervals of (0-4), (4-7), (7-24)

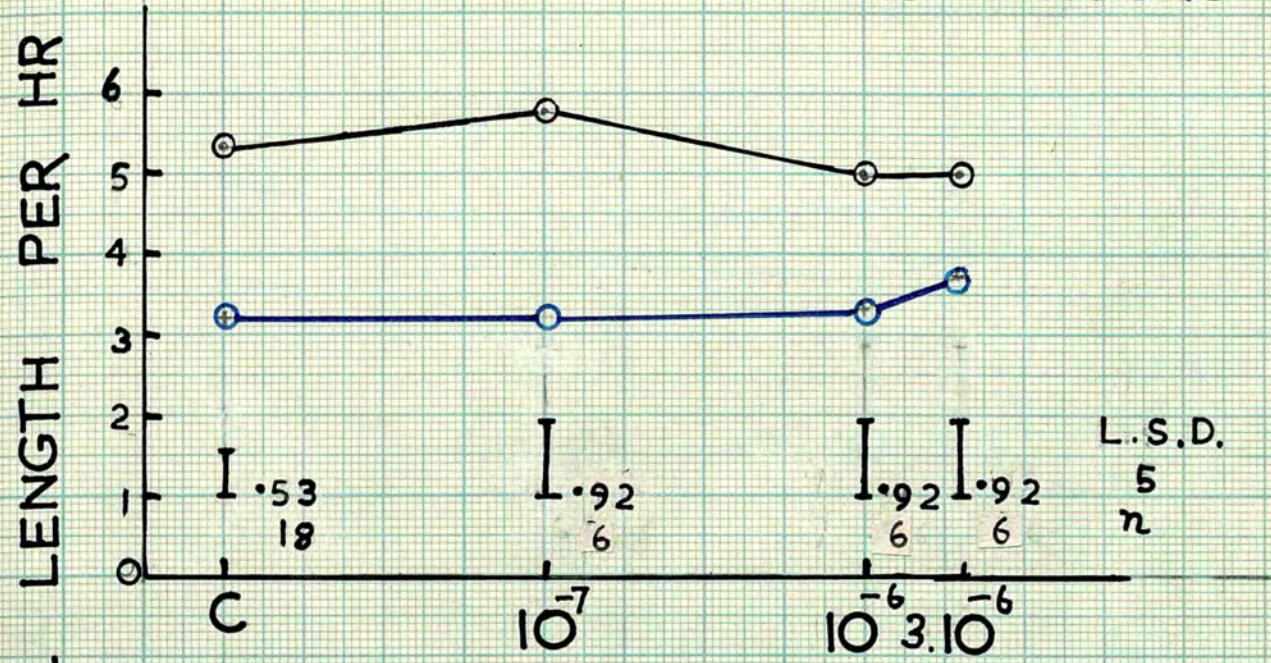


Figure 28

The graphs showing the interaction between P-chloro-phenoxy-iso-butyric acid (P.C.I.B.) and B-indolyl-acetic acid (IAA) (inhibitory concentration) in the extension growth of pea root sections. The times noted are the growth periods after excision from the parent root. The vertical lines represent the least significant differences between <sup>relevant</sup> means at 5% probability level.



0-4 HOURS



4-7 HOURS

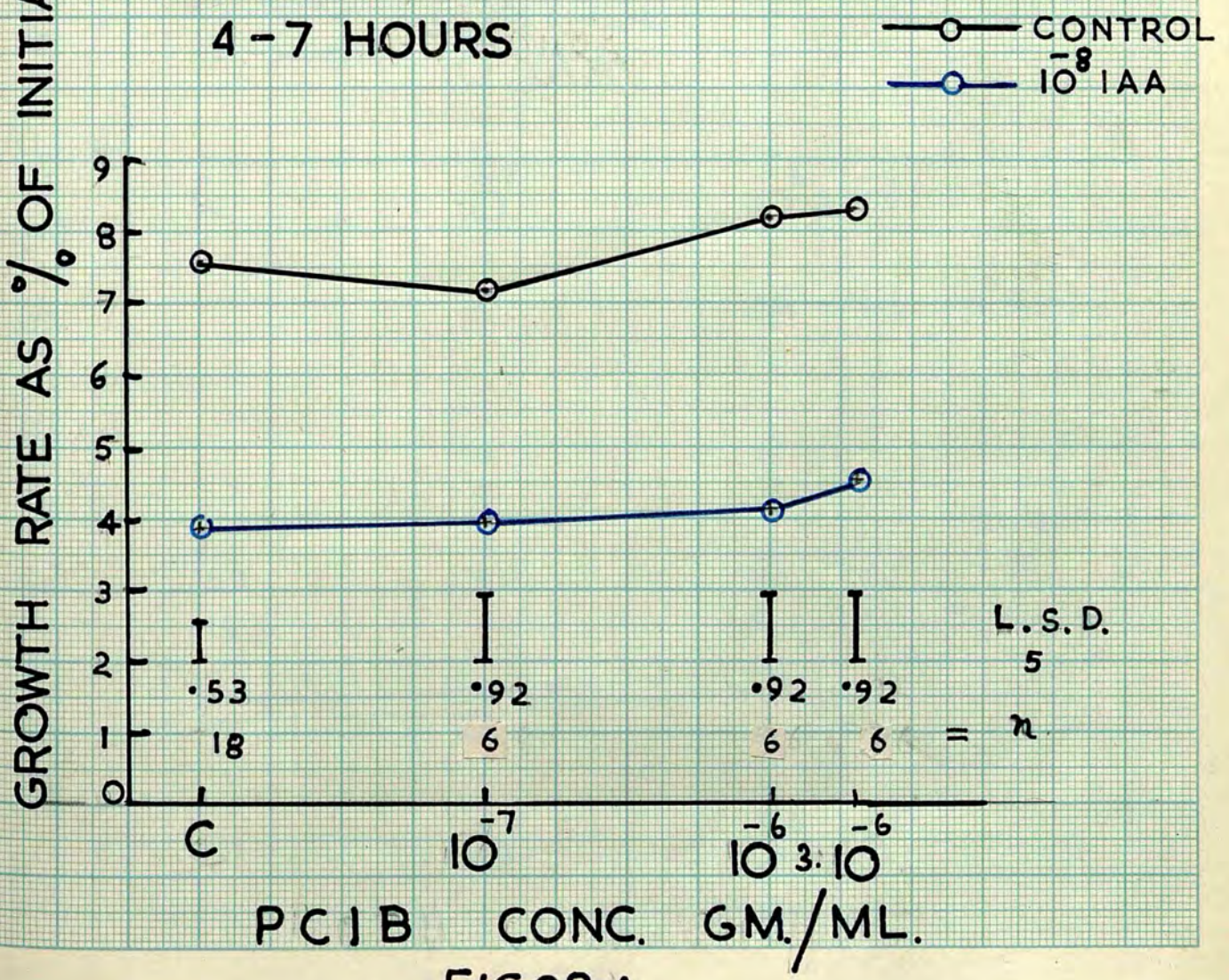


FIG 28 A



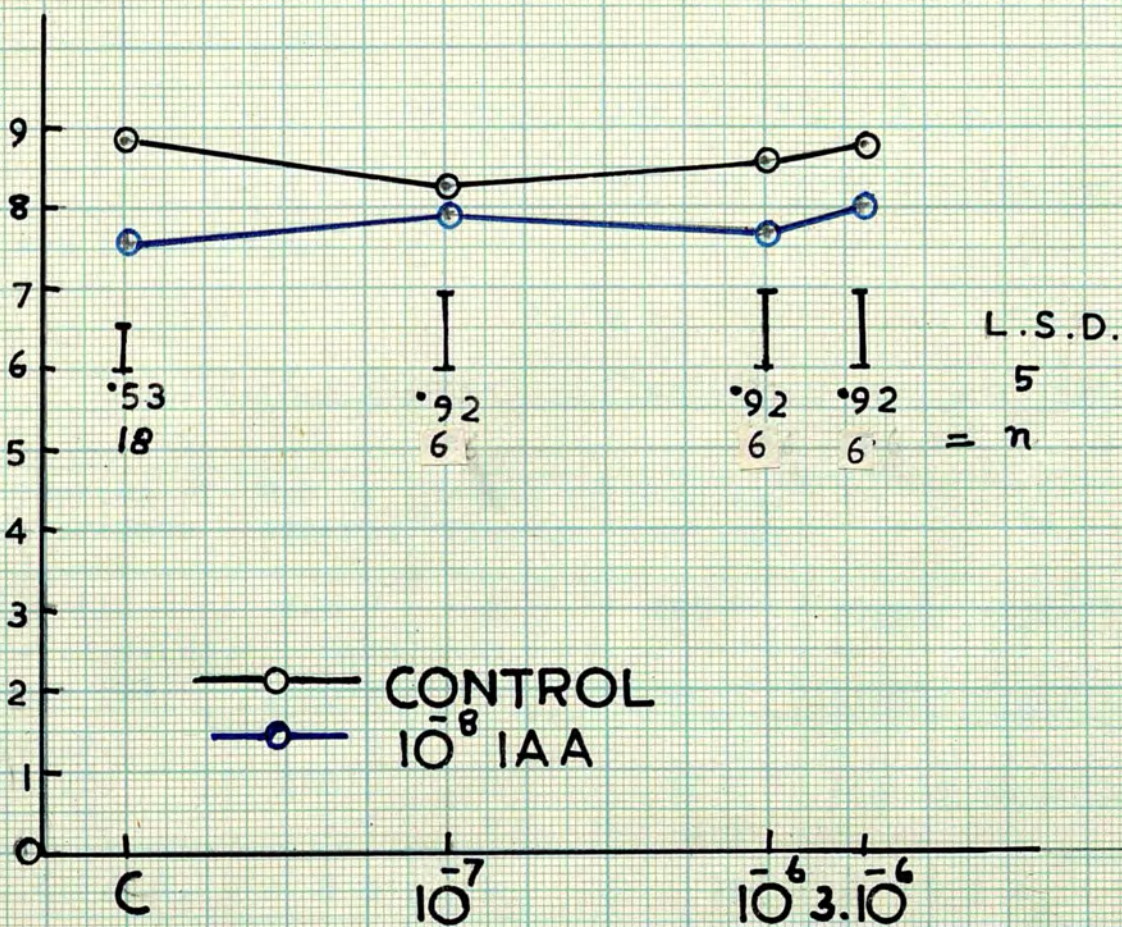
7-24 HOURS

LENGTH PER HR

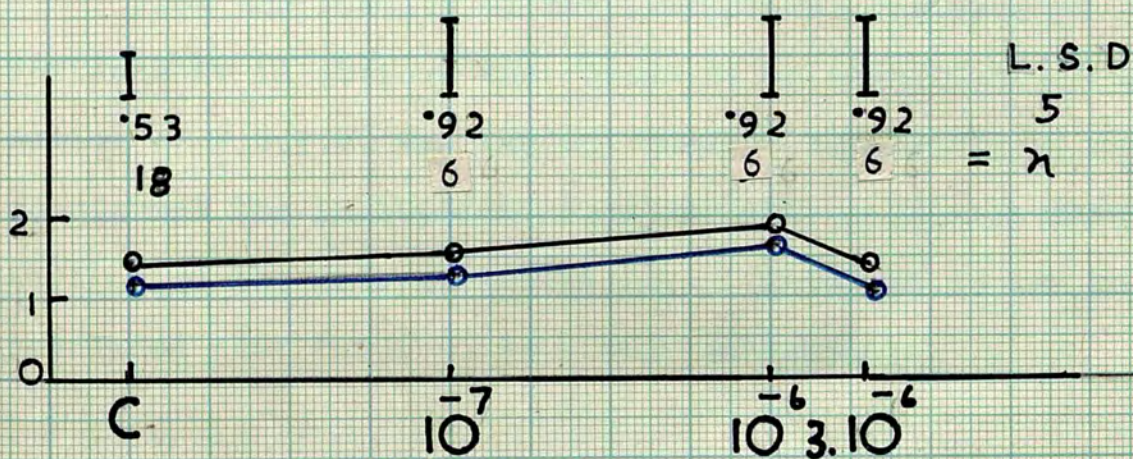
% OF INITIAL

GROWTH RATE AS % OF

7-24 HOURS



24-48 HOURS



P C I B CONC. GM/ML

FIG 28 B



and (24-48) hours. Then rates of growth were calculated for each interval.

The results are reproduced in (Fig. 28, A, B, \_\_\_\_\_).

A statistical analysis was performed and the results are shown below:

Table 57

P.C.I.B.-IAA Interaction (inhibitory concentration)

Sources of variance	Sum of squares	Degree of freedom	Mean sq. variance	P.
IAA (I)	205.7	1	205.7	highly significant.
P.C.I.B.(P)	2.1	3	.7	-
Time (T)	1760.2	3	586.7	highly significant.
I.X.P.	1.5	3	.5	-
I.X.T.	128.5	3	42.8	highly significant.
P.X.T.	9.9	9	1.1	-
I.X.P.X.T.	6.8	9	.76	-
Residual	163.7	256	.639	-

$$n = 18, \text{ L.S.D. } = \sqrt{\frac{1}{18}} \cdot 53$$

$$n = 6, \quad " \quad = \sqrt{\frac{1}{6}} \cdot 92$$

From the results of the statistical analysis of variance it is obvious that there is no significant interaction of P.C.I.B. and IAA which means that P.C.I.B. cannot counteract the inhibition caused by IAA. It also cannot antagonise the natural inhibitor. Therefore it can be concluded that P.C.I.B. is a weak antiauxin.



(III) Studies on the Interaction of IAA-C.N.B.

The same experimental procedure as for the preceding experiment, was adopted. Concentrations of C.N.B. from one part in  $10^7$  to one part in  $10^4$  were used in combination with two stimulatory concentrations of IAA, i.e. ( $10^{-11}$ ,  $10^{-10}$  gm/ml. The plan of the individual experiment is shown below:

Table 58

IAA-C.N.B. Interaction

Plan of individual experiment

conc. (gm/ml.) C.N.B.	Conc. (gm/ml.) IAA		
	0	$10^{-11}$	$10^{-10}$
0	2	2	2
(x) any one concentration	2	2	2

For each concentration of C.N.B. three such experiments were carried out and the growth was measured at (0-4), (4-7), (7-24) and (24-48) hours under a travelling microscope.

A plan of the full investigation is given below:

Table 59

IAA-C.N.B. Interaction

The plan of the full investigation.

Conc. (gm/ml.) IAA	Conc. (gm/ml.) C.N.B.				
	0	$10^{-7}$	$10^{-6}$	$10^{-5}$	$10^{-4}$
0	24	6	6	6	6
$10^{-11}$	24	6	6	6	6
$10^{-10}$	24	6	6	6	6

Figure 29

Graphs showing the interactions between B-indolyl-acetic acid (IAA) and 4-chloro-3-nitro-benzoic acid (C.N.B.) in the stimulation of extension growth of excised pea root sections. The times noted above are the growth periods after excision from the parent root. The vertical lines represent the least significant differences at the 5% probability level between the means of growth rates in the series of concentration combinations against which they are placed. The figures against the lines record the numbers of replicate samples from which each mean of the series was calculated.



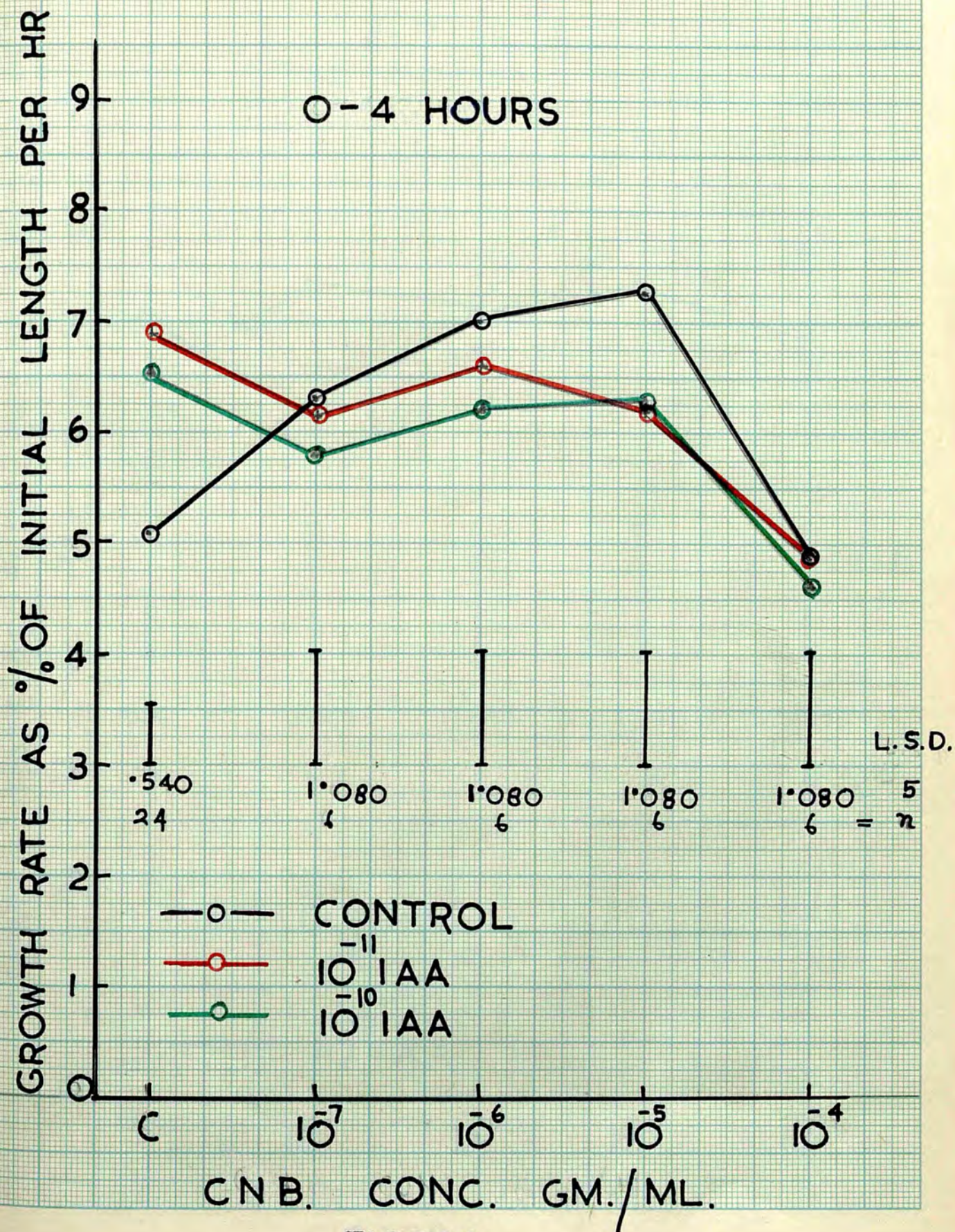
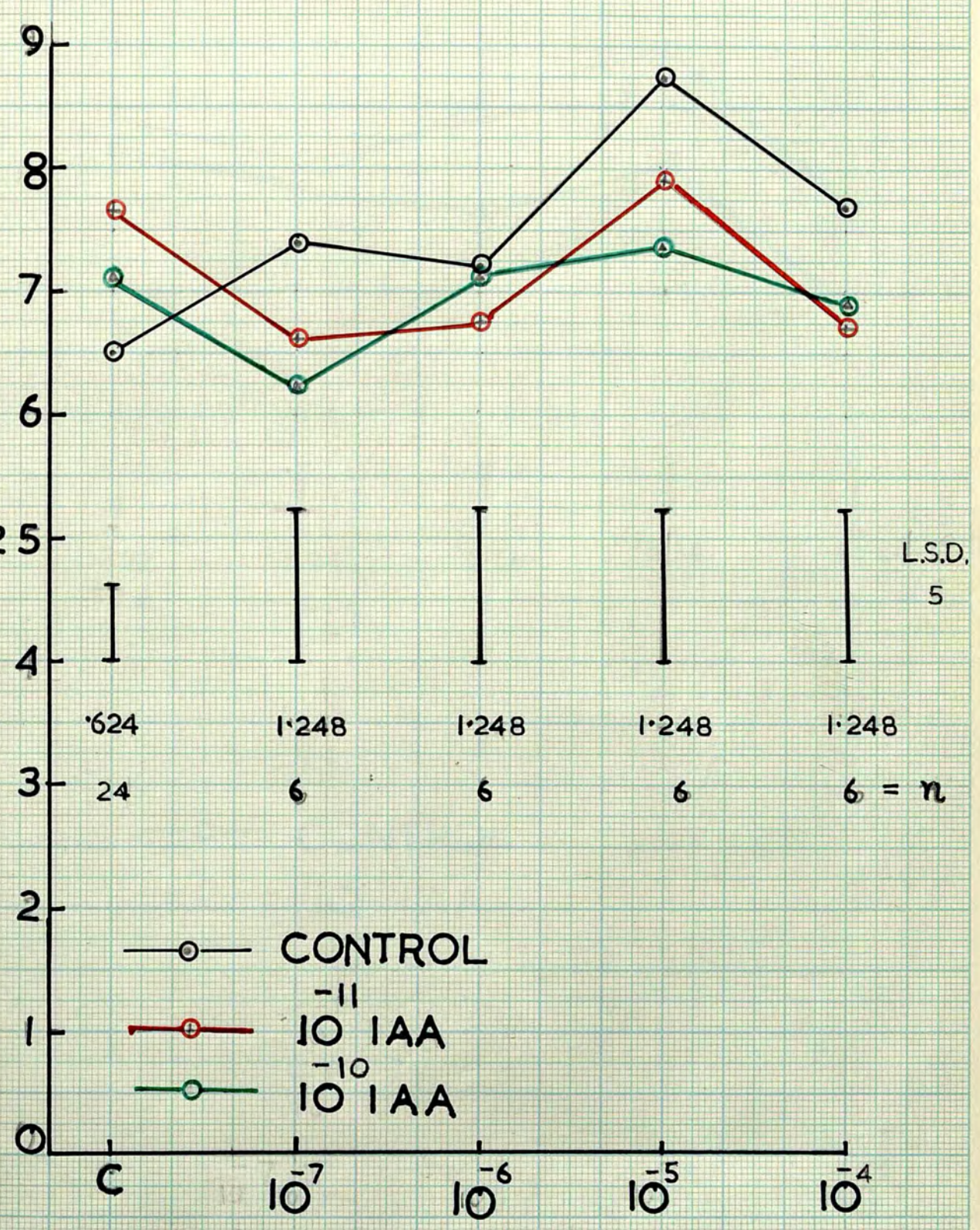


FIG 29A



GROWTH RATE AS % OF INITIAL LENGTH PER HR

4-7 HOURS



C N B CONC. G M./M L.  
FIG 29B



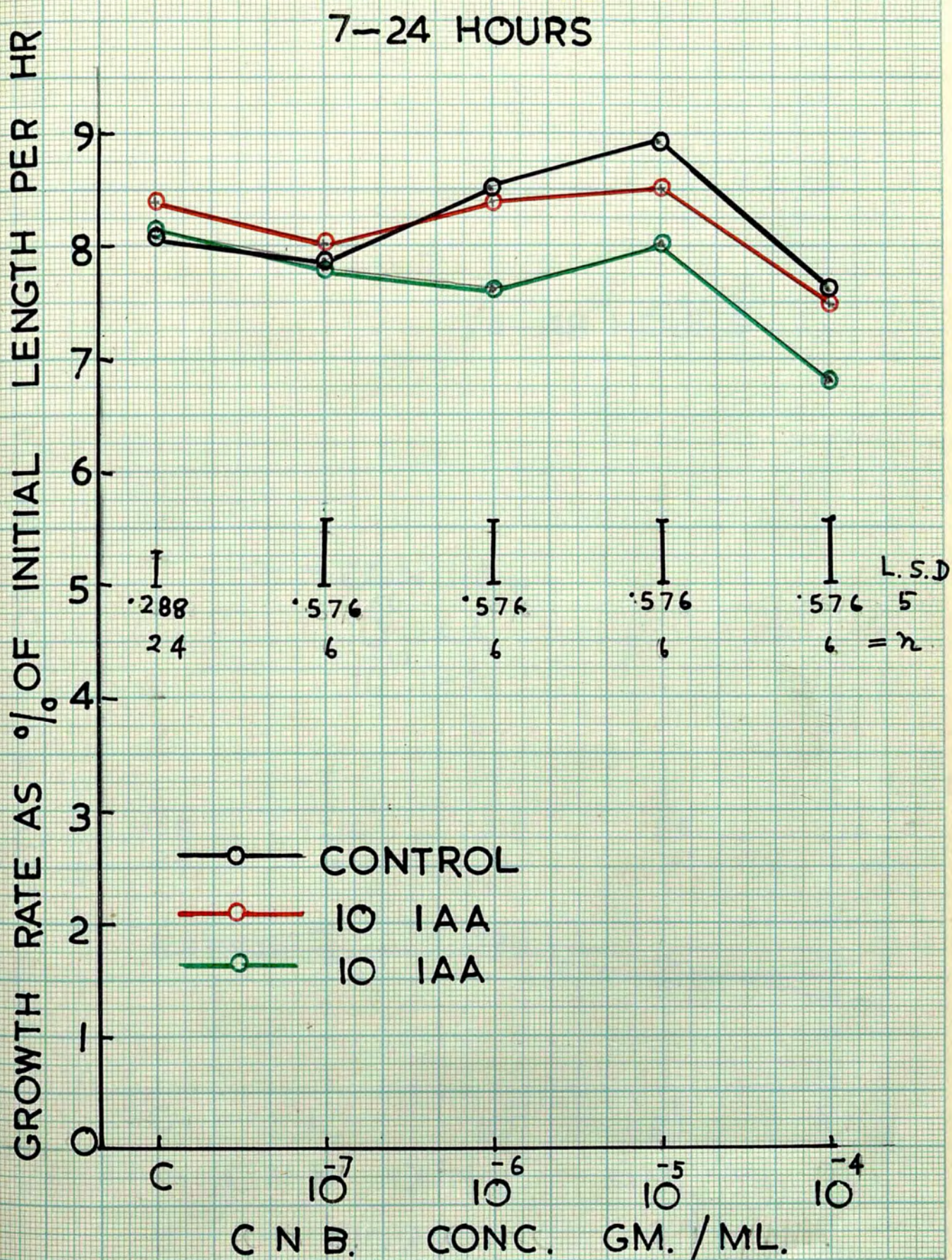


FIG 29C



GROWTH RATE AS % OF INITIAL LENGTH PER HR

24-48 HOURS

—○— CONTROL  
 —○—  $10^{-11}$  IAA  
 —○—  $10^{-10}$  IAA

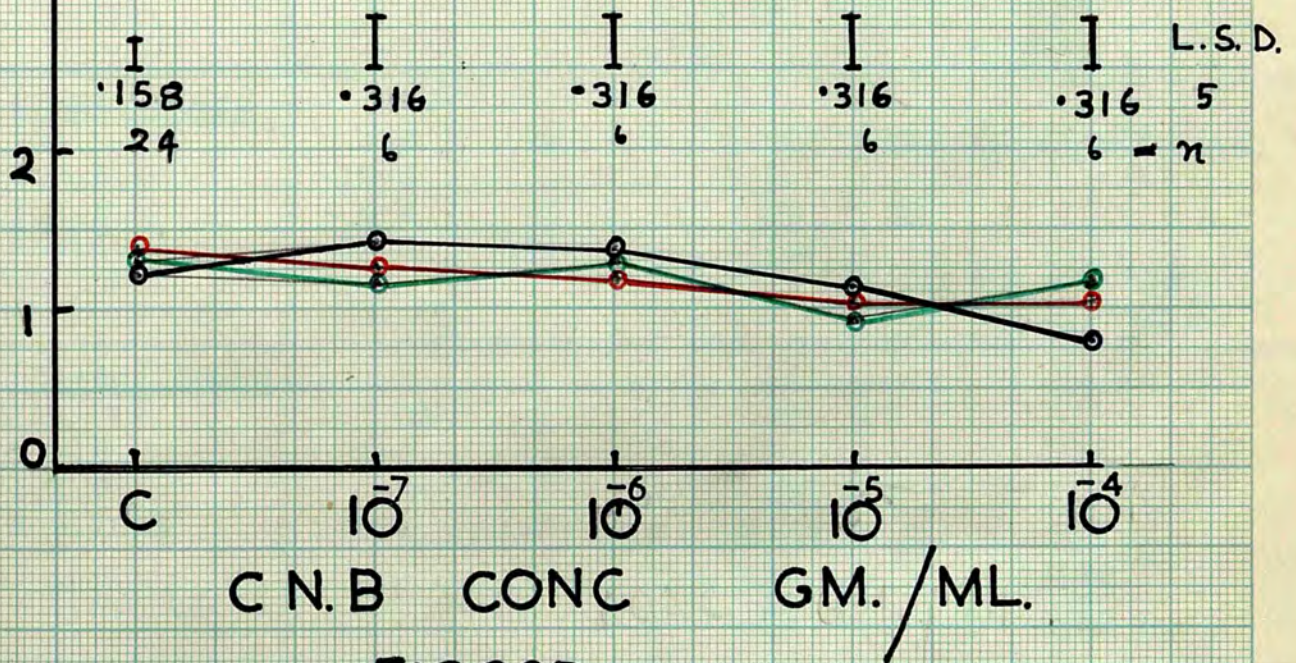


FIG 29D



The rates of growth (extension) per hour were calculated as percentage of the initial size of sections from the readings at different intervals.

The results are reproduced in (Fig. 29, A,B,C,D)

Table 60

IAA-C.N.B. Interaction

Sources of variance	Sum of squares	Degrees of freedom	Mean sq. variance	P.
<u>Time period (0-4) hours</u>				
IAA (I)	11.2	2	5.6	.1%
C.N.B.(B)	38.8	4	9.7	highly significant.
I.X.B.	37.6	8	4.7	" "
Residual	113.1	129	.876	
			n = 24, L.S.D. at 5% = .540	
			n = 6, " = 1.080	
<u>(4-7) hours</u>				
IAA	2.5	2	1.25	-
C.N.B.	16.7	4	4.17	1%
I.X.B.	28.4	8	3.55	.1%
Residual	149.8	129	1.161	
			n = 24, L.S.D. = .624	
			n = 6, " = 1.248	
<u>(7-24) hours</u>				
IAA	4.5	2	2.75	highly significant.
C.N.B.	15.2	4	3.8	" "
I.X.B.	4.4	8	.55	5%
Residual	32.2	129	.249	
				L.S.D. = .288
				.576
<u>(24-48) hours</u>				
IAA	.06	2	.03	-
C.N.B.	1.8	4	.45	-
I.X.B.	.94	8	.118	-
Residual	10.2	129	.0790	
			n = 24, L.S.D. = .158	
			n = 6, " = .316	

The highly significant triple interaction (I.X.B.X.T) necessitates a breakdown analysis with time. The results of the analysis of variance shows that the growth stimulation by C.N.B. is highly significant for first three periods, but disappears at (24-48) hours. Interaction of IAA and C.N.B. is highly significant at (0-4) hours, at (4-7) hours this interaction is significant at .1% level and at (7-24) hour period the interaction falls to 5% level, but in (24-48) hours interaction completely disappears. This indicates that the degree of interaction between IAA and C.N.B. tends to be less with progress of time. This interaction of IAA and C.N.B. takes the form of a mutual antagonism of stimulation. (Fig 29, A,B,C). Thus many of the combination of two stimulatory concentrations of IAA and C.N.B. respectively gave growth rates which were smaller than those with either of the two compounds alone, and at the same concentrations. In some combinations, e.g. IAA and C.N.B. at  $10^{-7}$  gm/ml. from (4-7) hours, growth rates were reduced to control level.

This mutual antagonism between (IAA) and antiauxins in their stimulatory effects on root section growth further supports the hypothesis that their physiological actions are identical.



(IV) Studies on the interaction of IAA-N.M.S.A. using sub-optimal concentrations

The mutual antagonism between auxin (IAA) and anti-auxin becomes well-marked when the concentrations of the two compounds are optimum. This suggests one possibility which needs serious attention. A combination of two optimal concentrations might show a false mutual antagonism between them since their combined effects might bring the effective concentration into an inhibiting range. A combination of two sub-optimal concentrations would not do this. Taking this point into consideration the interaction of auxin and antiauxins using sub-optimal concentrations was studied.

The action curves of IAA and N.M.S.A. which are shown in (Fig. 16, Fig. 18 ) show that the sub-optimal concentration of IAA is  $2 \times 10^{-12}$  gm/ml and that of N.M.S.A. is  $2 \times 10^{-6}$  gm/ml. These two concentrations were used in combination in the following experiments:

Table 61

The plan of the experiments is

Conc. (gm/ml.) N.M.S.A.	Conc. (gm/ml.) IAA	
	0	$2 \times 10^{-12}$
0	6	6
$2 \times 10^{-6}$	6	6

Figure 30

Graphs showing the interaction of B-indolyl-acetic acid (IAA) and 1-naphthyl-methyl-sulphide-acetic acid (N.M.S.A.) and its variation with time in the stimulation of extension growth in excised pea root sections. The concentrations of both compounds were suboptimal. The vertical lines show the least significant difference between means at the 5% probability level.



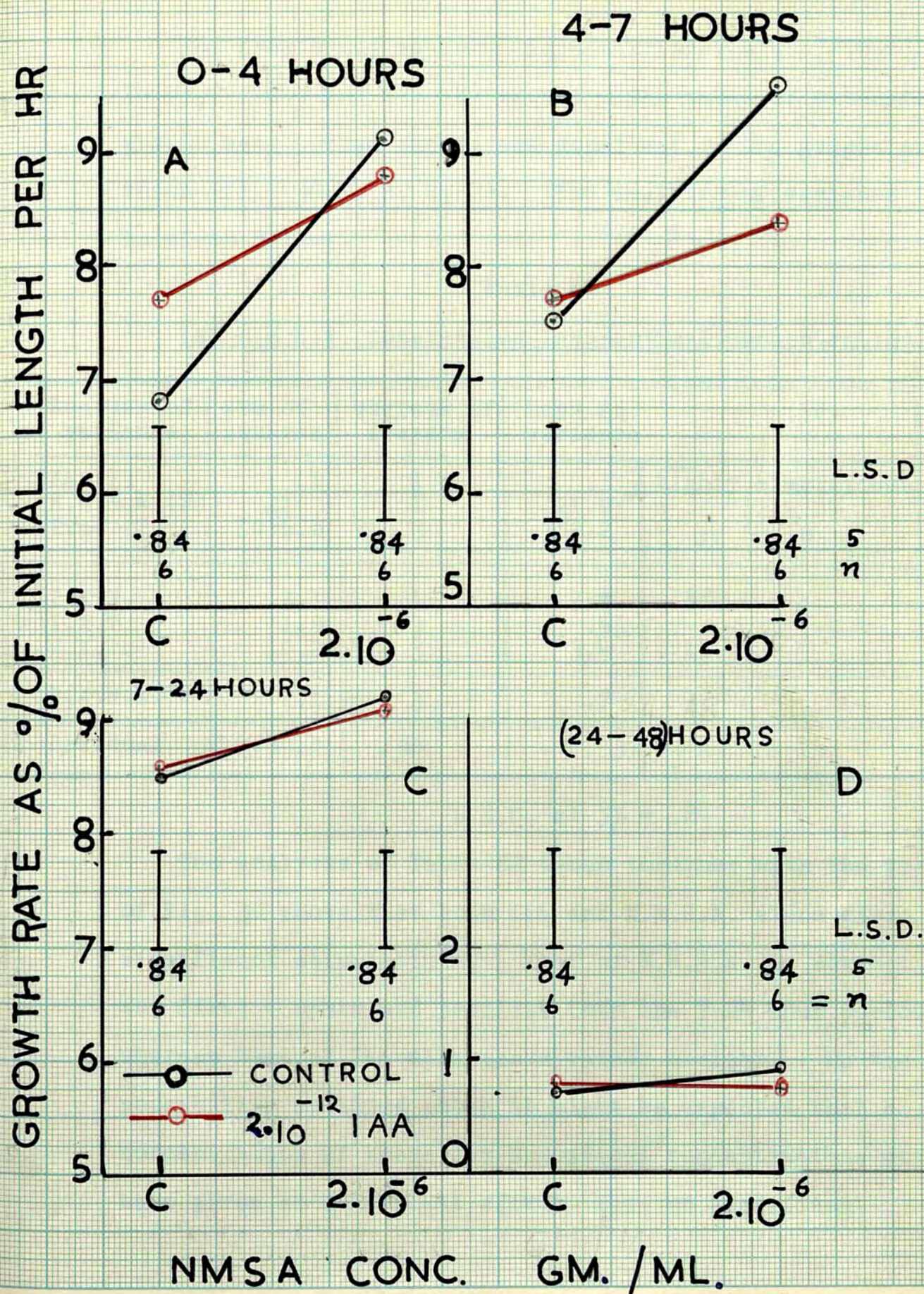


FIG 30



The growth was measured in usual intervals of (0-4), (4-7), (7-24) and (24-48) hours. The rates of extension growth were worked out in the usual way. The graphs are shown (Fig. 30 A, B, C, D). The analysis of variance table is given below:

Table 62

IAA-N.M.S.A. interaction (sub-optimal concentration)

Sources of variance	Sum of squares	Degrees of freedom	Mean sq. variance	P.
IAA (I)	.1	1	.1	highly significant ,, "
N.M.S.A. (N)	21	1	21.0	
Time (T)	1056.8	3	352.2	
I.X.N.	3.1	1	3.1	5%
I.X.T.	1.9	3	.63	-
N.X.T.	10.2	3	3.4	. 1%
I.X.N.X.T.	2.1	3	.7	-
Residual	43.0	80	.537	

L.S.D. at 5% = .84

The analysis of Table 62 taken together with the graphs of Fig. 30 shows that both IAA and N.M.S.A. alone give significant stimulations. That of N.M.S.A. is maximal in the first 4 hours and persists into the 7-24 hour period whereas that of IAA disappears rather earlier. On the possibility outlined above it might be expected that a concentration of these two suboptimal concentrations might have given an additional effect, i.e. no significant



interaction variance. This significant interaction which was obtained is due to a general mutual antagonism in which the response to mixtures of these two substances was intermediate between the response to either one acting alone. This therefore rules out the possibility of the previous antagonism being a false antagonism caused by combinations of two optimal concentrations of growth stimulants.

#### 6. The mutual interaction of the antiauxins

The mutual antagonism between auxin (IAA) and anti-auxins in their stimulatory effects on root section growth further supports the hypothesis that their physiological actions are identical. This possibility should naturally be explored further by studying the mutual interactions of the antiauxins themselves.

##### (I) The interaction of N.M.S.A. and C.N.B.

The interaction of two antiauxins N.M.S.A. and C.N.B. was studied. Only stimulatory concentrations of both the compounds were used.

The stimulatory concentrations ranging from one part in  $(10^6 - 10^4)$  of N.M.S.A. were used in all possible combinations with concentrations of C.N.B. from one part in  $(10^6 - 10^4)$ .

The plan of the individual experiment is shown on the next page:

Table 63

N.M.S.A.-C.N.B. Interaction

## Plan of Individual Experiment

Conc. (gm/ml.) C.N.B.	Conc. N.M.S.A. (gm/ml.)				
	0	$10^{-6}$	$10^{-5}$	$5 \times 10^{-5}$	$10^{-4}$
0	2	2	2	2	2
(x) any one conc.	2	2	2	2	2

For each concentration of N.M.S.A. three experiments were performed.

The plan of the full investigation is given below:

Table 64

N.M.S.A.-C.N.B. Interaction

## Plan of Full Investigation

Conc. (gm/ml.) C.N.B.	Conc. (gm/ml.) N.M.S.A.				
	0	$10^{-6}$	$10^{-5}$	$5 \times 10^{-5}$	$10^{-4}$
0	24	6	6	6	6
$10^{-6}$	24	6	6	6	6
$10^{-5}$	24	6	6	6	6
$10^{-4}$	24	6	6	6	6

The growth (length) of sections was measured over the usual periods of (0-4), (4-7), (7-24) and (24-48) hours. Precisely the same procedure was adopted as for the preceding



Figure 31

Graphs showing the interactions between 1-naphthyl-methylsulphide-acetic acid (N.M.S.A.) and 4-chloro-3,nitrobenzoic acid (C.N.B.) in the stimulation of extension growth of excised pea root sections. The times noted above are the growth periods after excising from the parent root. The vertical lines represent the least significant differences at the 5% probability level between the means of growth rates in the series of concentration combinations against which they are placed. The figures against the lines record the numbers of replicate samples from which each mean of the series was calculated.



GROWTH RATE AS % OF INITIAL LENGTH PER HR

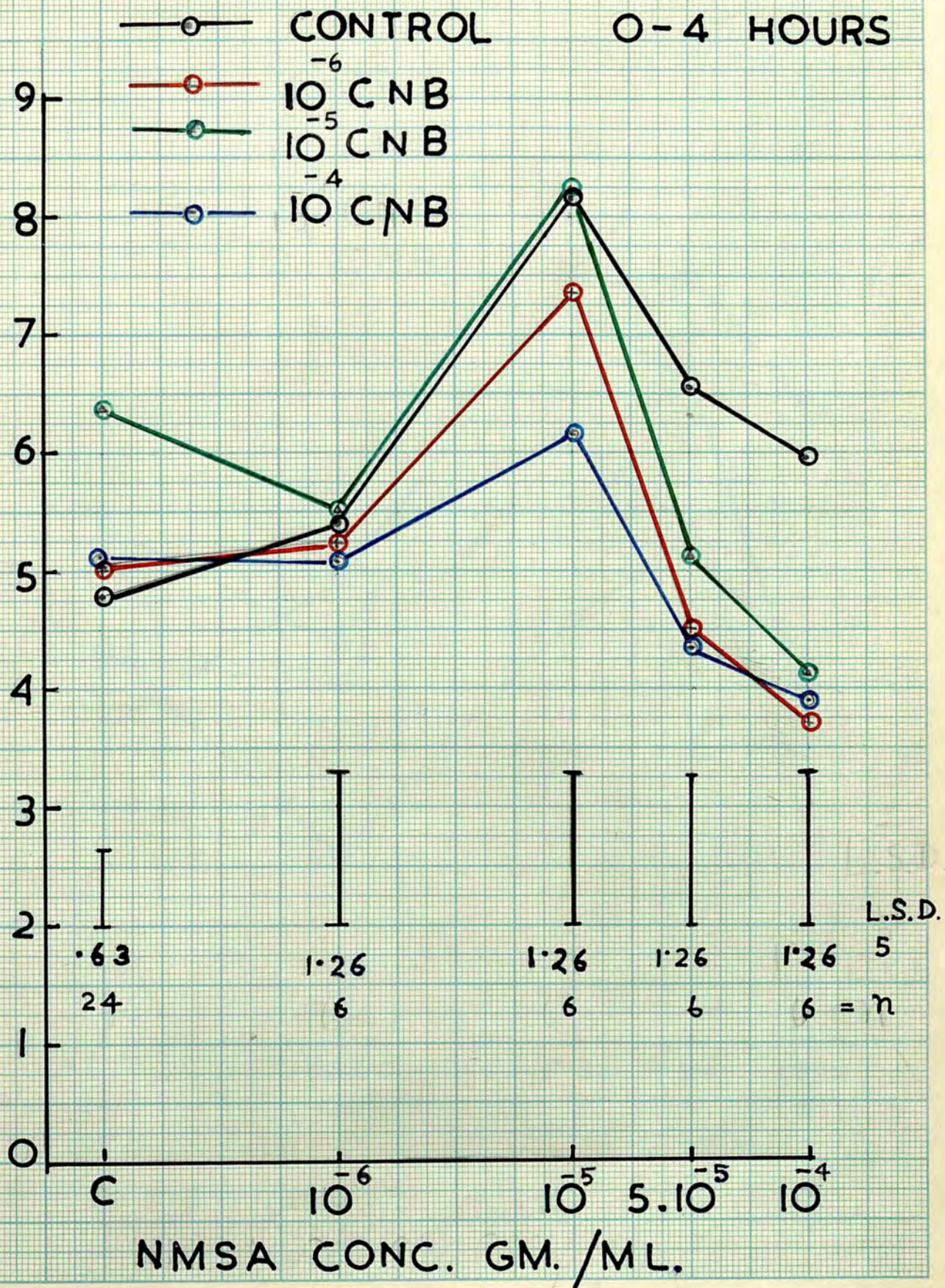


FIG3IA



GROWTH RATE AS % OF INITIAL LENGTH PER HR

4-7 HOURS

—○— CONTROL  
 —○—  $10^{-6}$  C.N.B  
 —○—  $10^{-5}$  C.N.B  
 —○—  $10^{-4}$  C.N.B

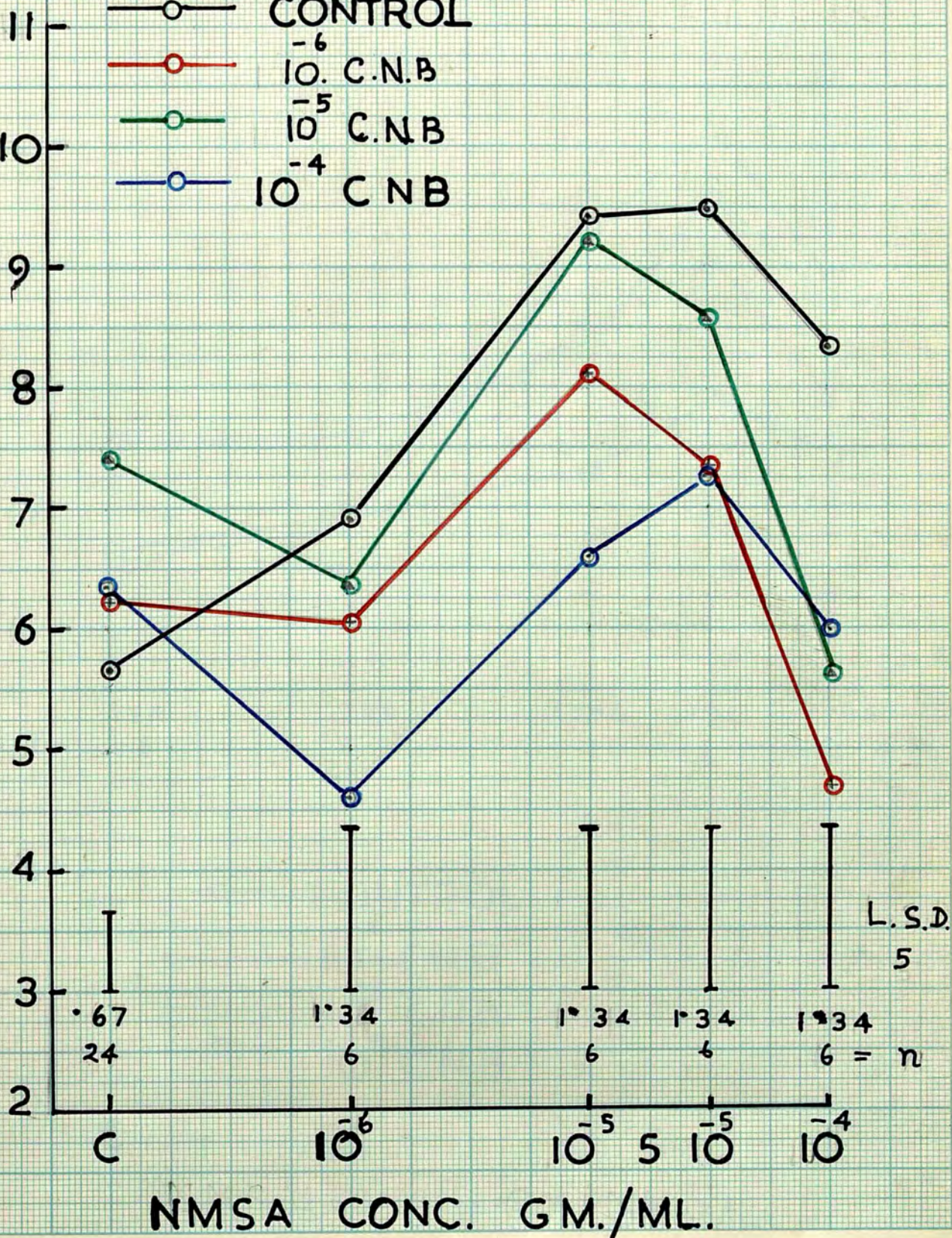
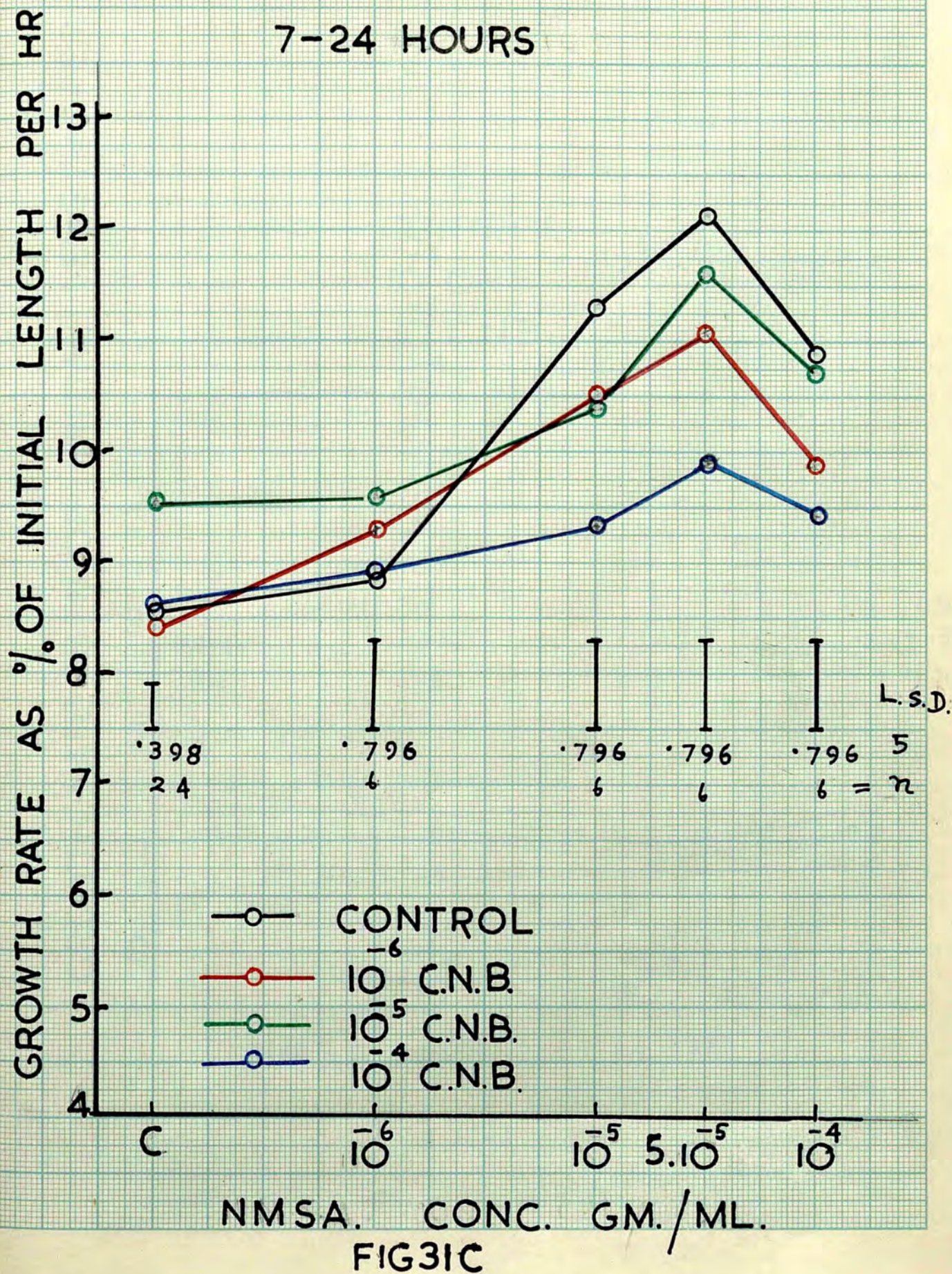


FIG3IB



7-24 HOURS





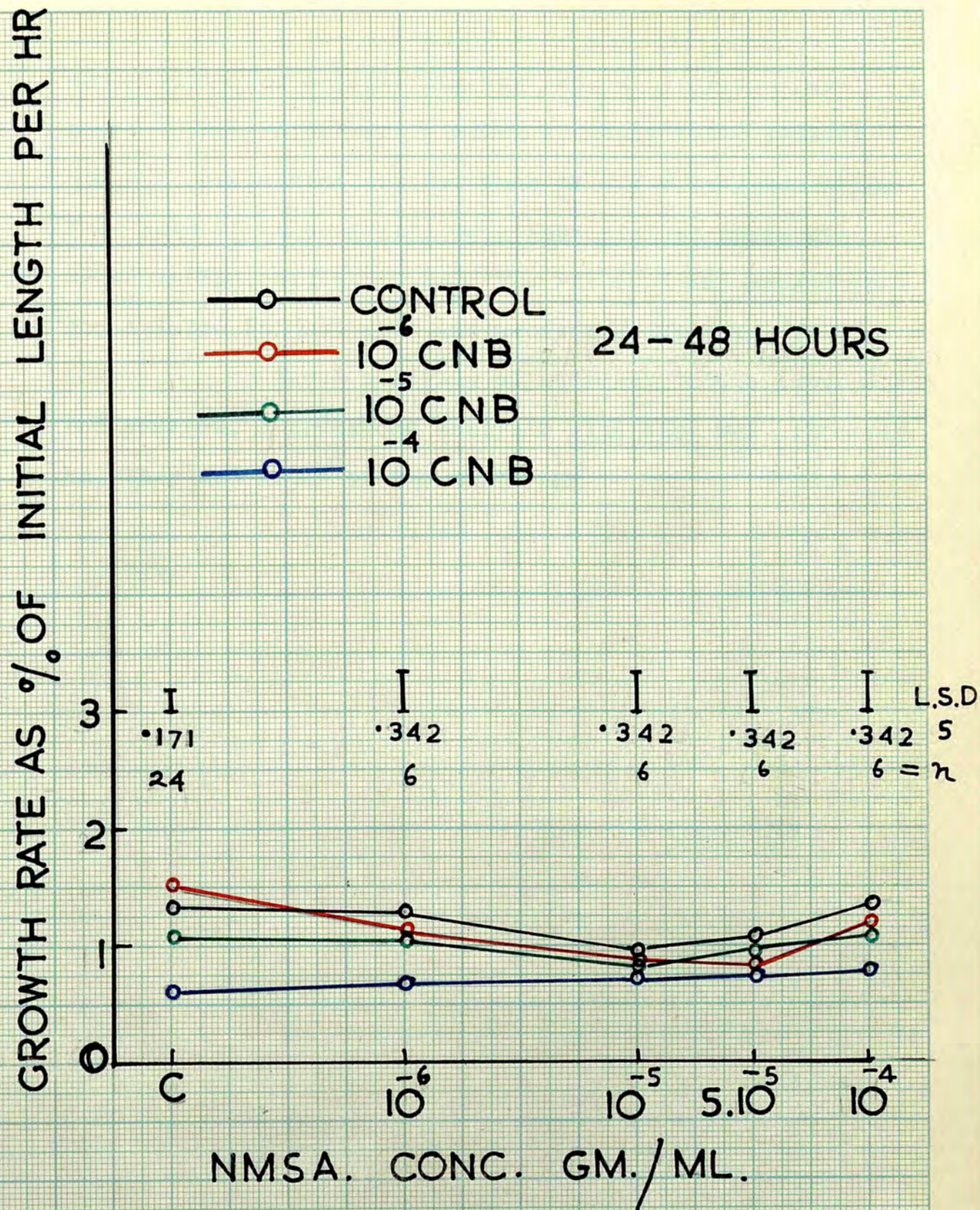


FIG 31D



experiments for calculating the extension growth per hour. The graphs were plotted at these rates against N.M.S.A. concentrations for each of C.N.B. concentrations. Four sets of three graphs were thus plotted which are shown in (Fig. 31, A,B,C,D.) The rates of the growth were accumulated in one table and necessary analysis was performed. Since a highly significant triple interaction had emerged, a breakdown analysis with time was performed and they are shown on the next page :

## (4-7) 4000

N.M.S.A.

143.8

4

25.4

Highly Significant

C.N.B.

45.8

8

15.06

S.T.B.

108.4

16

8.75

Residual

837.1

278

1.88

## (7-24) 1000

N.M.S.A.

131.4

4

40.4

Highly Significant

C.N.B.

83.9

8

11.3

S.T.B.

24.9

16

8.24

Residual

58.1

178

4.7

## (24-45) 5000

N.M.S.A.

1.4

4

8

C.N.B.

1.4

8

2.2

S.T.B.

2.4

16

4.2

Residual

18.3

178

4.2



Table 65

N.M.S.A.-C.N.B. Interaction

Sources of variance	Sum of squares	Degrees of freedom	Mean Sq. variance	P.
<u>Time period</u>				
<u>(0-4) hours</u>				
N.M.S.A.(N)	127.6	4	31.9	highly significant.
C.N.B.(B)	32.1	3	10.7	" "
N.X.B.	55.7	12	4.64	0.1%
Residual	204.6	172	1.19	
				L.S.D. 5% n = 24, L.S.D. = .63 6 " = 1.26
<u>(4-7) hours</u>				
N.M.S.A.	143.5	4	35.9	highly significant
C.N.B.	45.2	3	15.06	" "
N.X.B.	105.	12	8.75	" "
Residual	237.1	172	1.38	
				L.S.D. = .67 = 1.35
<u>(7-24) hours</u>				
N.M.S.A.	161.6	4	40.4	highly significant
C.N.B.	33.9	3	11.3	" "
N.X.B.	26.9	12	2.24	" "
Residual	82.1	172	.477	
				L.S.D. = + .398 + .796 -
<u>(24-48) hours</u>				
N.M.S.A.	1.9	4	.47	-
C.N.B.	10.	3	3.33	at 5%
N.X.B.	3.6	12	.3	-
Residual	15.3	172	.0889	
				L.S.D. = .178 = .342

This analysis of variance shows that the effects of N.M.S.A. and C.N.B. are highly significant in the first three periods, but are not significant in the last phase of growth. These results suggest that the penetration of both compounds into the cells is very rapid. The interaction of two compounds is highly significant over those first three periods (0-4), (4-7) and (7-24). The interaction in the last phase is not significant, due possibly to the fact that these two substances alone have no effect during this phase. This interaction between N.M.S.A. and C.N.B. takes the form of a mutual antagonism of stimulation of precisely the same nature as that of C.N.B.-IAA interactions.

(II) Interaction between P.C.I.B. and N.M.S.P.

Experiments on a simpler plan than those above were performed to study the interaction of two antiauxins P.C.I.B. and N.M.S.P.

In this investigation one part in  $10^6$  of P.C.I.B. was used with two concentrations of N.M.S.P., i.e. one part in  $10^5$  and one part in  $10^4$ . The plan of an individual experiment is given on the next page:



Table 66

P.C.I.B. and N.M.S.P. Interaction

Plan of individual experiment

Conc. (gm/ml.) P.C.I.B.	Conc. (gm/ml.) N.M.S.P.		
	0	$10^{-5}$	$10^{-4}$
0	3	3	3
$10^{-6}$	3	3	3

P.C.I.B.-N.M.S.P.-Interaction

Plan of full investigation

Table 67

Conc. (gm/ml.) P.C.I.B.	Conc. (gm/ml.) N.M.S.P.		
	0	$10^{-5}$	$10^{-4}$
0	18	9	9
$10^{-6}$	18	9	9

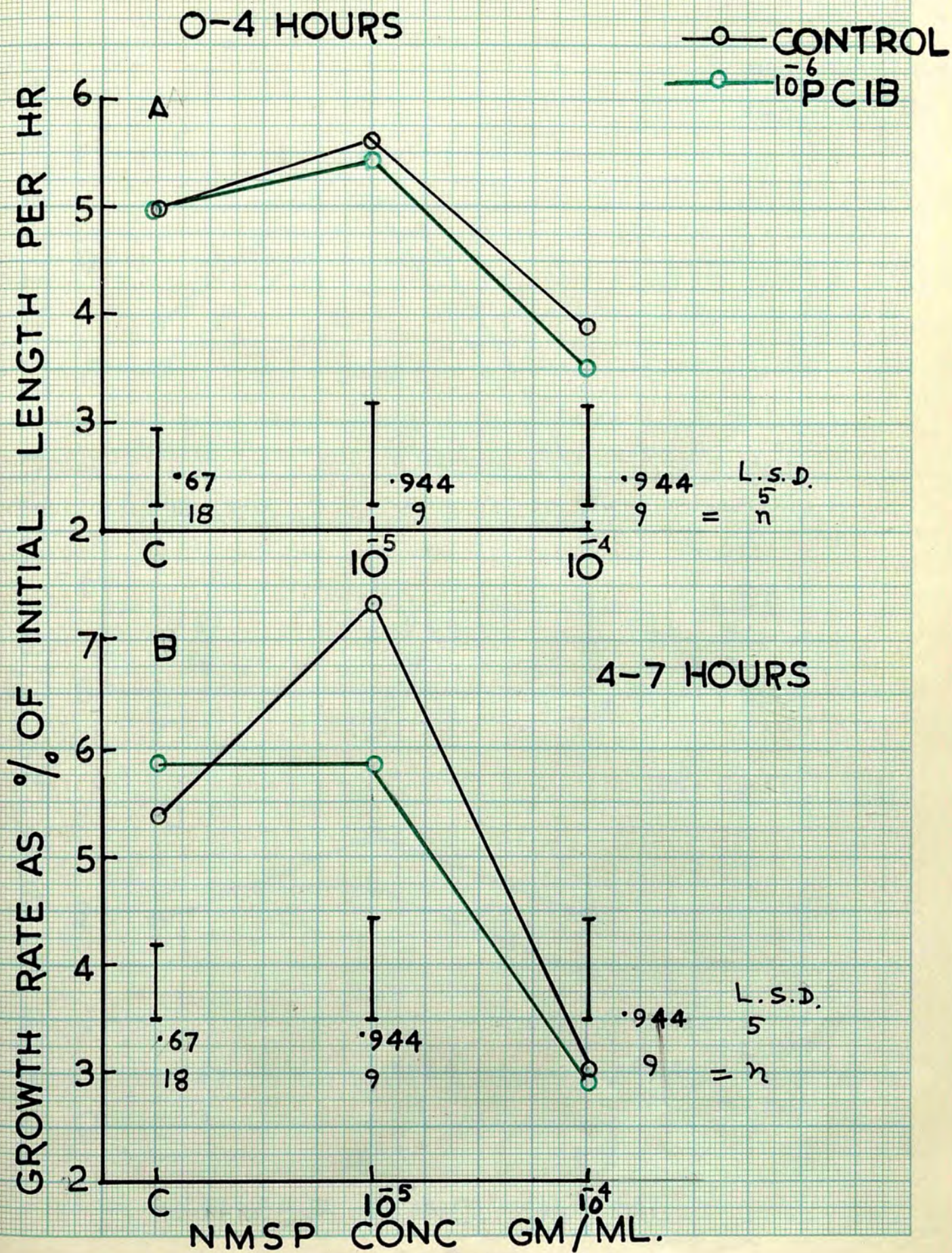
The graphs at different intervals of time, (0-4), (4-7), (7-24) and (24-48) are shown in (Fig. 32) A+B.

The analysis of variance table is shown on the next page:

Figure 32

Graphs showing the interactions between a (1-naphthyl-methyl-sulphide) propionic acid (N.M.S.P.) and p-chlorophenoxy-iso-butyric acid (P.C.I.B.) in the stimulation of extension growth of excised pea root sections. The times noted above are the growth periods after excising from the parent root. The vertical lines represent the least significant differences at the 5% probability level between the means of growth rates in the series of concentration combinations against which they are placed. The figures against the lines record the numbers of replicate samples from which each mean of the series was calculated.







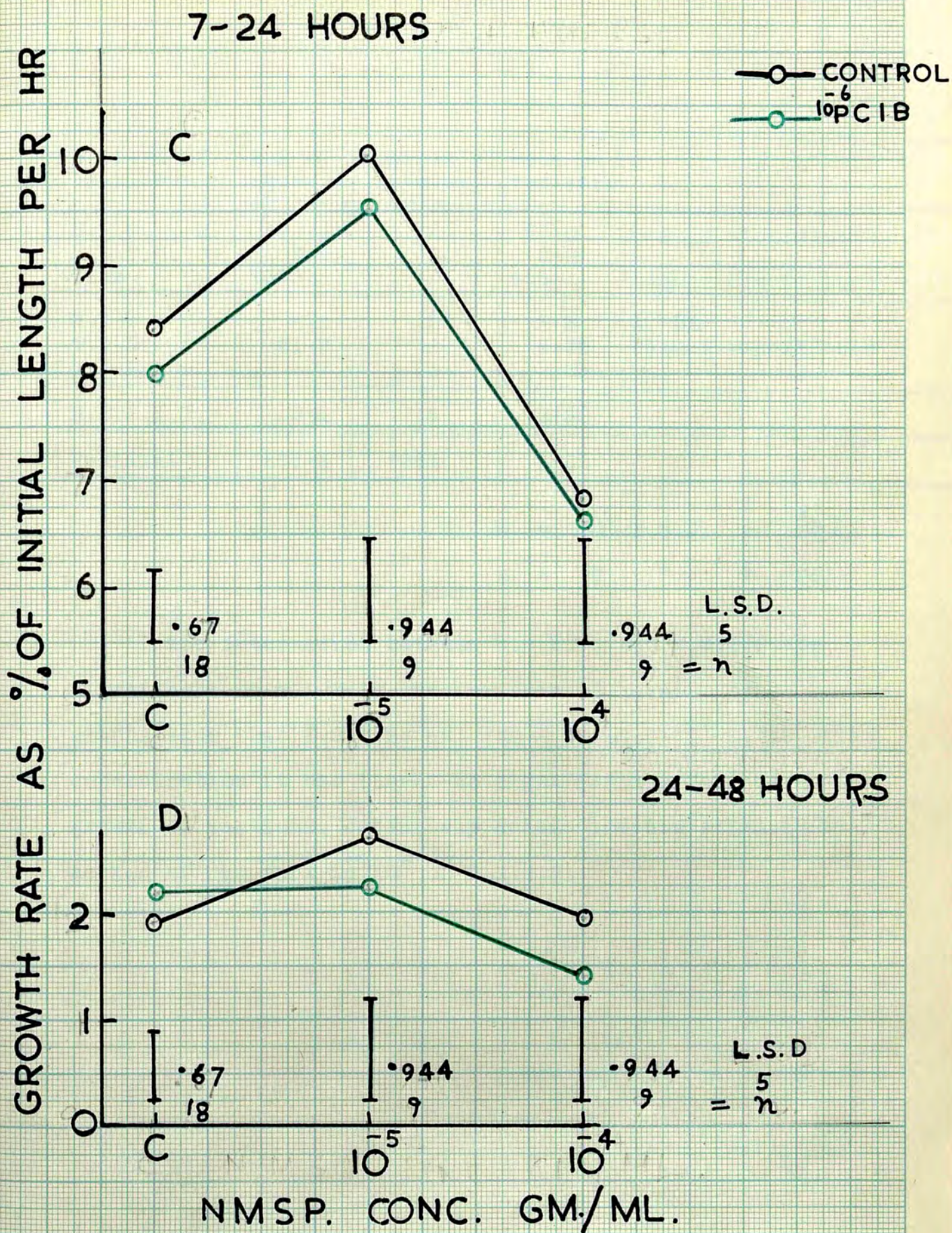


FIG 32 B



Table 68

P.C.I.B.-N.M.S.P. Interaction

Sources of variance	Sum of squares	Degrees of freedom	Mean sq. variance	P.
N.M.S.P.(N)	195.6	2	97.8	highly significant.
P.C.I.B.(P)	2.9	1	2.9	-
Time	1368.3	3	456.1	highly significant.
N.X.P.	6.6	2	3.3	$\leq 5\%$
T.X.N.	51.2	6	8.53	highly significant.
T.X.P.	.7	3	.23	-
N.X.P.X.T.	10.1	6	1.68	-
Residual	266.2	*264	1.01	

$$n = 18 \quad \text{L.S.D.} = .67$$

$$n = 7 \quad " \quad \dagger \quad .944$$

The interaction between N.M.S.P. and P.C.I.B. is significant at slightly greater than 5% level. The graphs show that P.C.I.B. at  $10^{-6}$  gm/ml. , at which concentration it has itself no significant effect on the growth of sections, definitely antagonises the stimulatory action of one part in  $10^5$  of N.M.S.P.

## CHAPTER V

Discussion and Conclusion  
=====

It will be seen from the experimental results presented in this thesis that both in their individual stimulatory and inhibitory effects on root section extension growth and in their mutual interactions, there is nothing to distinguish in principle one compound from another in the group of five already tested. Eventually, the conclusion to be drawn is that whatever the situation in shoots and coleoptiles and in inhibition of root growth, these auxins and antiauxins cause stimulation of root growth by precisely the same physiological mechanism. And hence the usually accepted idea that antiauxins stimulate root-growth by antagonising an endogenous auxin present in supra-optimal concentrations does not seem to be consistent with the results obtained here.

Audus and Shipton (1952) suggested that this proposed common physiological action of auxin and antiauxins might be the antagonism of a natural endogenous inhibitor present in the root. This was only one of the several possibilities. The other one, for example, would be that the two types of compounds may have a direct stimulatory action on the growth system of roots, in precisely the same sort of way that auxin is supposed to stimulate shoots and coleoptiles. The data presented in this thesis largely enable us to evaluate which one of the two views would have more weight. A good deal of



work on the relationships between growth response and external concentration (C) of growth substances in both coleoptiles and roots has been done by Kaindl (1951), McRae and Bonner (1953), Hellstrom (1953), Bennet-Clark and Kefford (1954). They suggest that they can all be closely related by a formula of the following type :-

$$\text{Response} = \frac{K.C.}{A + B.C.}$$

where K, A and B are constants.

Now if  $B = 0$ , this takes the form of the equation for enzyme kinetics, which has been shown to fit with remarkable closeness the growth response of coleoptile sections to applied IAA. (McRae and Bonner 1953, Bennet-Clark and Kefford 1954). If  $A = 1$  and  $K = B$  this equation will have an expression directly derivable from Freundlich adsorption isotherm, where the response would be directly proportional to the concentration of growth substance adsorbed at some cell interface. Hellstrom (1953) has shown that it closely fits in with the phenomenon of double response to added growth substances to roots (i.e. stimulation followed by inhibition at higher concentrations), and the antagonistic interaction of auxins and antiauxins in the inhibition of root. The assumption was made that growth substances affect growth as a result of their adsorption at some particular site in the cell (see also

Kaindl 1951). This adsorption centre may be an enzyme surface or it may not. There is no way of distinguishing between these two alternatives merely from the shapes of growth response curves.

Hellstrom in the treatment of his data took into consideration more than one <sup>adsorbates</sup> competing for the same adsorption centre. The results presented in this thesis can be considered in the light of his treatment of multiple adsorption pattern. An attempt will be made to explain the interaction of the applied growth substances according to the two theories mentioned above.

According to the first theory (Audus and Shipton, 1952), the stimulation of root growth is caused by an antagonism of a natural endogenous inhibitor. If this is so, there are three adsorbates in the system, the natural inhibitor and two reacting exogenous antagonists of this inhibitor, which are, say, A and B. At low concentration, when the two antagonists are not supposed to have direct action on growth, the growth response will be proportional to the quantity of inhibitor "pushed off" the centres where it is adsorbed and exerting its inhibition, i.e. the growth inhibitor will be pushed off  $(1-\alpha)$ , where  $\alpha$  is equal to the fraction of the adsorption points occupied by the inhibitor. It can be shown (unpublished analysis by Professor L. J. Audus) that the combination of the two growth substances will always result



Figure 33

Theoretical interaction curves for two growth substances active in the stimulation of extension growth of root sections, assuming growth control to be exerted by such substances when adsorbed at a specific growth centre.

- A) When the stimulation is produced by the competitive antagonism of a natural endogenous growth inhibitor.
- B) When the stimulation is a direct action at the growth centre. (Fig. 33A and B are due to Professor L.J. Audus)

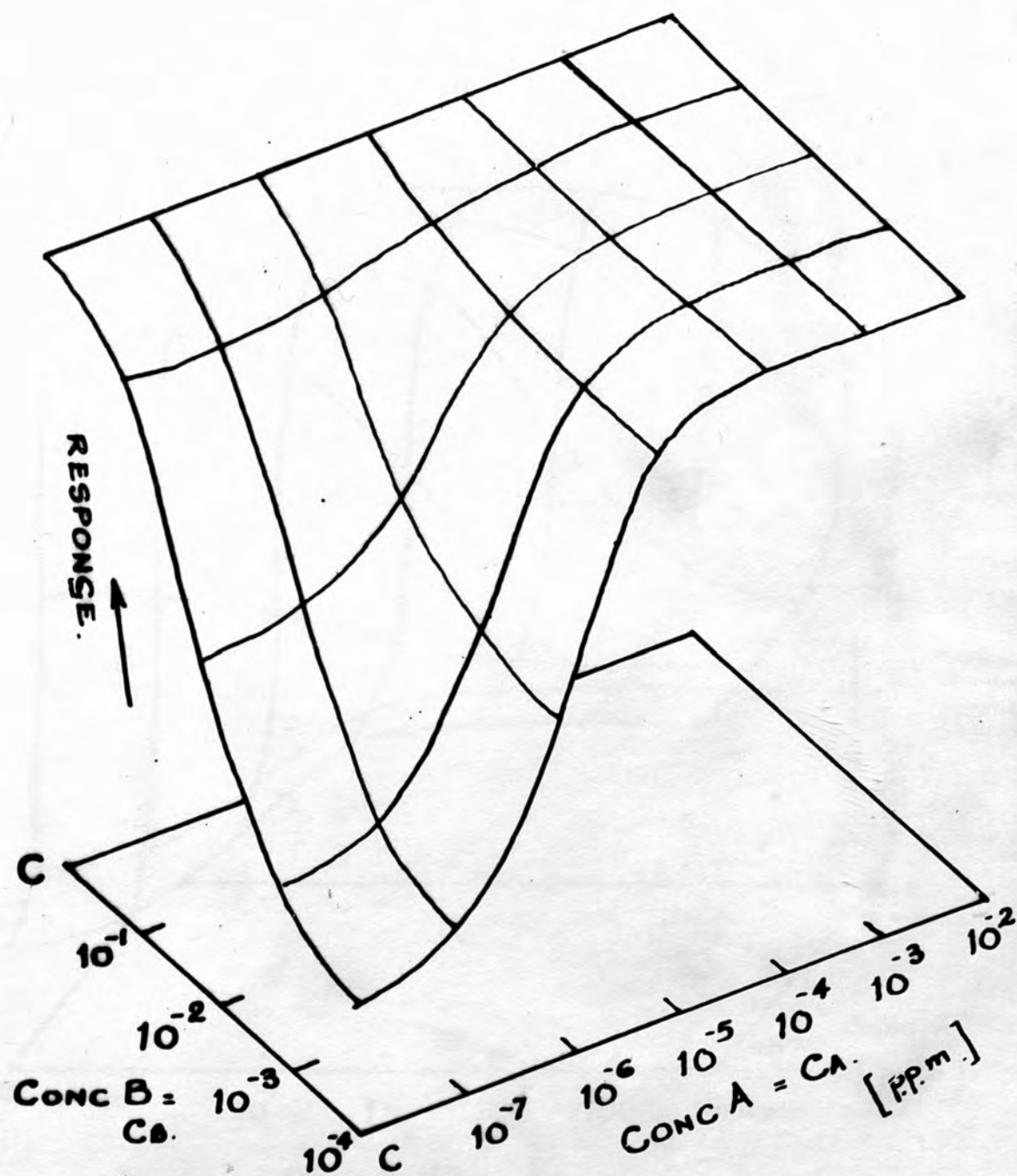


FIG 33 (A)



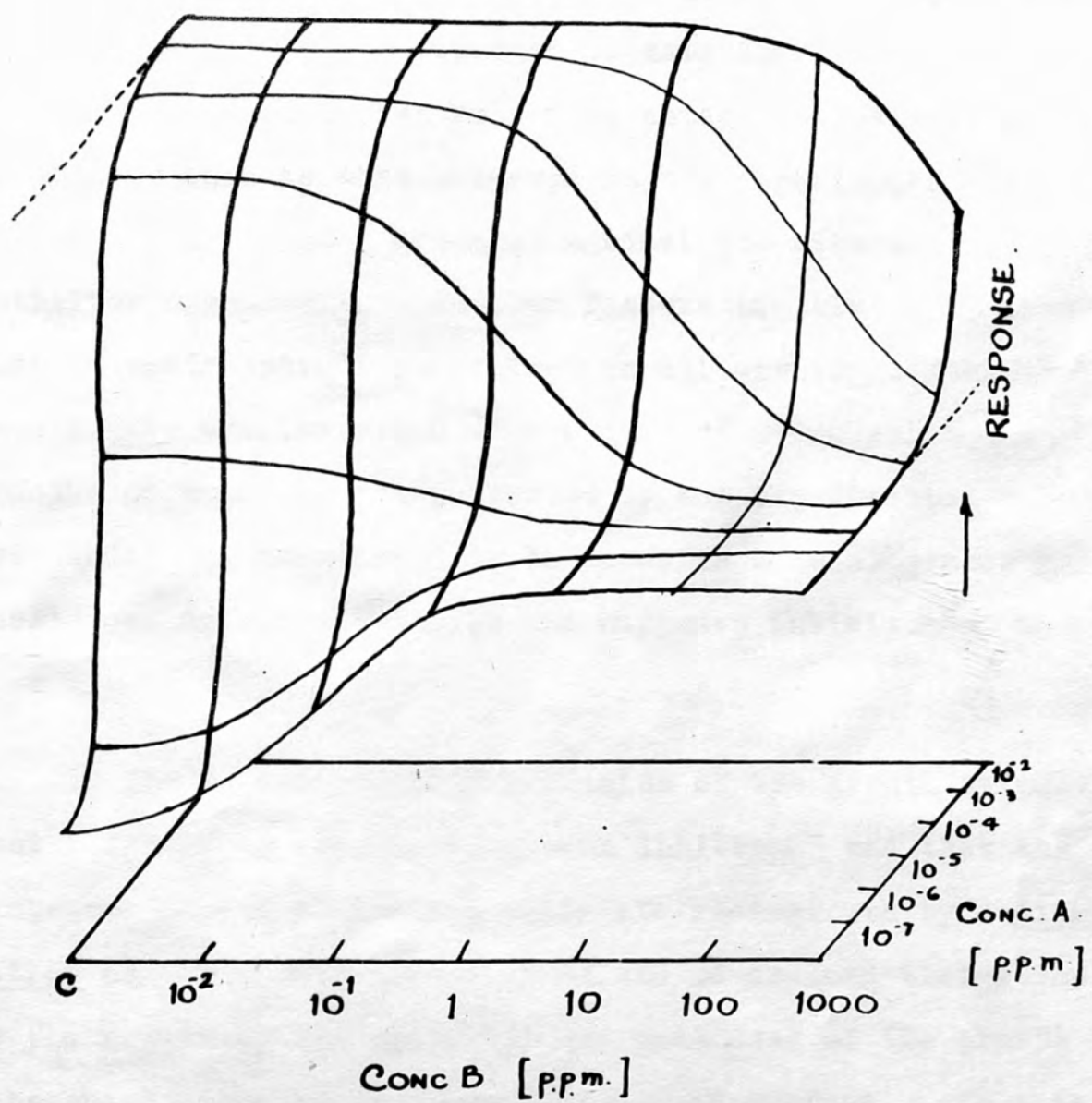


FIG 33 (B)

in an effect greater than that of either acting alone at the same concentrations, but there is an upper limit beyond which stimulation will not go when all the inhibitor has been "pushed off" its centre by either or both of antagonists A or B. (Fig. 33 A) due to Professor L. J. Audus, shows the interaction of A and B expected on this theory.

There should not be mutual antagonism between growth substances such as that observed in the experiments above and this is a very strong argument against the natural endogenous inhibitor hypothesis. Another feature of this hypothesis is that at their optimum concentration all active substances should give precisely the same degree of stimulation - a conclusion which is not supported by any experimental results. Eventually the conclusion to be drawn is that exogenous growth substances do not antagonise the supposed inhibitor of the root growth.

The second possible mechanism of the growth stimulation is that there is an endogenous growth inhibitor and that the exogenous growth substances stimulate root growth by a direct action on the growth system. It can be assumed that stimulation is the result of the action of the molecules of the growth substances adsorbed at some protoplasmic surface. In this hypothesis there will be two adsorbates A and B. A and B will occupy the fractions of the area in the adsorption pattern  $a_A$  and  $a_B$  respectively: (from Hellström formula 1953).



Now from the studies on the interaction of auxins and antiauxins in the growth of root Åberg (1952) has shown that the growth substance molecules have two distinct properties:

- 1) An "affinity" for the growth centre,
- 2) An "activity" at the growth centre.

Using these basic properties he has shown that auxins with high "affinity" and low "activity" can behave as efficient antagonists against more active auxins. On this basis the two growth substances A and B, we have considered, will have activity  $A_A$  and  $A_B$ , and their growth response will be proportional to the products  $a_A A_A$  and  $a_B A_B$ . The total growth response due to the mixture in that case will be proportional to  $a_A A_A + a_B A_B$ , when it is assumed that the "activities" of either A or B are not modified by the presence of the other molecule. (Fig. 33B, due to Professor L.J. Audus) shows the theoretical interaction between compounds A and B in root growth stimulation expected on this theory, where the "affinity" of A is  $10^4$  times that of B and the "activity" of A  $2\frac{1}{2}$  times that of B. Here it is clear that there is a definite antagonism of the action of the "stronger" compound A by the "weaker" one B. As the concentration of B is increased so the magnitude of the joint effect of A+B is "pulled down" towards the lower stimulation level corresponding to saturation of the growth centre by B. It will be seen that for any

combination of concentrations the magnitude of the net effect on growth falls somewhere between the effects of the two compounds acting singly at those concentrations. It can never be smaller than that of the weakest growth substance acting alone.

On the whole this picture fits the results obtained, particularly with those substances giving immediate growth responses, i.e. IAA ~~X~~ C.N.B, and N.M.S.A ~~X~~ C.N.B. In (Fig. 29,) it is clear that a combination of IAA at both stimulatory concentrations with optimum concentrations of C.N.B. give effects that are of the same magnitude as the smaller effect given by IAA alone. Here IAA is the weaker growth substance (corresponding with B) antagonising the stronger C.N.B. (corresponding with A). In precisely the same way in (Fig. 31) C.N.B. as the weaker growth substance is seen to antagonise the action of the stronger N.M.S.A. It should be noted that if  $A = A_B$ , i.e. if the two growth substances have identical "activities" then this second surface assumes virtually the same form as the first and the two theories are indistinguishable.

The above scheme, although it explains well most of the data, leaves certain points which cannot be explained by it. For example, the combinations of low (sub-optimal) concentration of the growth substances with high (optimal) concentrations of the weak growth substance giving much lower growth responses than either acting alone, i.e. a marked mutual antagonism.



It is clearly seen in combinations of  $10^{-6}$  gm/ml N.M.S.A. with ( $10^{-6}$ ,  $10^{-5}$  and  $10^{-4}$ ) gm/ml C.N.B. (Fig. 31). It is also seen, although not so clearly marked, in the IAA X C.N.B. interaction with IAA at ( $10^{-11}$  and  $10^{-10}$ ) gm/ml and C.N.B. at  $10^{-7}$  gm/ml and  $10^{-6}$  gm/ml. (Fig. 29).

The results of the experiments described in this thesis give very strong evidence that the mutual antagonism is a real one.

It is difficult to visualise what really is responsible for this "mutual antagonism".

Mere competition for a common growth centre of action is not sufficient to explain it. It may be that the theoretical assumption made regarding the "activities" ( $A_A$  and  $A_B$ ) of the competing substances is not enough to explain this kind of interaction between growth substances. If the "activity" of a molecule were in some way reduced by the presence of a competing molecule on a neighbouring adsorption site, then an explanation can be furnished for this excessively low response to these mixtures.

We cannot of course rule out the possibility that the mutual antagonism can be one of entry into the sections. If such entry involved an adsorption at an interface and if the compounds competed for this interface then results on growth rate such as those described might be obtained. On the whole, however, this possibility is not favoured since pretreatment

with one compound to ensure entry did not prevent the action of that compound being antagonised by another compound added subsequently.

### Conclusion

Two major conclusions have emerged from the foregoing experimental results:

- 1) They give very strong support to the earlier postulates that, as root growth stimulators, both auxins and their homologous antiauxins are functioning in identical ways in the same growth system.
- 2) They also indicate that the idea of stimulation by antagonism of endogenous growth inhibitor may have to be abandoned, since the interacting patterns suggest a direct action of these compounds at the growth centres.

If these principles prove, on extension of studies to a wider range of compounds, to be of general application, then the current notion concerning the molecular structural requirements of growth substances active on roots may have to be modified. It is quite possible that the rules drawn from the studies of coleoptiles and stem sections will not be strictly applicable to the growth substances active in root extension growth. A quite different set of structural requirements may ~~will~~ be required.



## SUMMARY

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(1) Investigation of the factors causing variation in the degree of stimulation by IAA in the growth of root sections

An examination has been made of the factors responsible for the wide variation in the stimulatory concentrations of IAA observed by earlier workers in this laboratory (Bedford College). These factors may be: effect of age of the seedlings; effect of diffuse light in which sections were cut and weighed; and effect of metallic impurities in ordinary distilled water.

(a) Age of root from which sections were cut

- (i) The age of the root from which the sections are cut has a marked effect on their total extension growth. The sections cut from 2 and 3 day old seedlings showed maximum total extension growth, while those from 4 and 5 day old ones gave much smaller growth.
- (ii) It has been shown that the stimulation and inhibition due to IAA do not change with the age of the root from which sections were taken and therefore slight difference in the age of the parent roots can not account for the sensitivity variations.

(b) Effect of light

Exposure to light during the growth period causes a

stimulation of about 10% in the growth of sections. In light the optimum IAA concentration is shifted from  $10^{-11}$  to  $10^{-10}$  gm/ml IAA. The degree of stimulation by IAA is not altered but apparent effectiveness of IAA is reduced suggesting a photo-inactivation of applied IAA.

Pretreatment with light prior to IAA application does not cause any change in the stimulatory effects of IAA. Manganese does not seem to affect the apparent photo-inactivation of IAA at least in roots. This is contrary to the findings of Galston in stem tissues.

Since the variations previously observed were in degree of stimulation at the optimum concentration ( $10^{-11}$  gm/ml) and not a shift in this optimum it is unlikely that variation in the lighting condition could account for the observed variations in sensitivity of root sections to IAA.

(c) The effects of metallic impurities likely to be present in ordinary distilled water

The investigation of the effects of metallic impurities were carried out by using special glass distilled water.

- (i) Calcium causes a stimulation of the growth of sections of about 4% at  $10^{-5}$  M. It does not alter growth response of sections to IAA.
- (ii) Manganese seems to stimulate the growth of sections at  $10^{-6}$  -  $10^{-5}$  M. No consistent interaction with IAA was observed. Further experiments would have to be performed to elucidate precisely the effect of manganese on IAA response.



- (iii) There is no stimulation of section growth by zinc. At  $10^{-4}$  M it causes inhibition of about 20%. There is an indication of an antagonism of IAA stimulation at  $10^{-5}$  -  $10^{-4}$  M. ~~zn~~
- (iv) Boron at concentrations of  $10^{-4}$  -  $10^{-3}$  M causes a slight inhibition of section growth. Boron at  $10^{-4}$  -  $10^{-3}$  M seems to antagonise the stimulation caused by IAA.
- (v) Cobalt causes a well marked stimulation of the growth of sections by about 14% at an optimum concentration of  $10^{-5}$  M. At  $10^{-4}$  M the inhibition is about 10%. There is a clear cut mutual antagonism of stimulation between IAA and cobalt.
- (vi) About 50% inhibition of growth is caused by copper at  $10^{-5}$  M. There is no interaction with IAA.

It can finally be concluded that, since the concentrations of metallic impurities likely to exist in ordinary distilled water are much lower than any of the above concentrations of metals giving interference with IAA stimulation, variations in the quantities of metallic impurities in ordinary distilled water are not likely to be the cause of the previously observed variations in sensitivity to stimulatory concentrations of IAA.

(2) Interaction of IAA and antiauxins in the stimulation of root growth

Investigations have been made into the effect on section growth of various combinations of stimulatory concentrations of

of IAA with stimulatory concentrations of certain antiauxins to check the theory of Audus and Shipton that such stimulations are the result of the antagonism of a natural endogenous root growth inhibitor.

(a) The effect of growth substances acting alone

(i) The action curve of IAA shows a marked difference in the growth response with each particular phase of section growth. The stimulation caused by  $10^{-11}$  gm/ml IAA is of the order of 20 - 25 % in the first four hours, falls to about 10 - 15 % in the next 3 hours and disappears over the next 20 hours. Concentrations above  $10^{-9}$  gm/ml inhibit the growth of sections.

(ii) N.M.S.P. stimulates the growth of sections in concentrations from  $10^{-8}$  -  $10^{-5}$  gm/ml. The degree of stimulation increases progressively up to 30% as the concentrations of N.M.S.P. increase from  $10^{-8}$  -  $10^{-5}$  gm/ml but at a concentration of  $10^{-3}$  gm/ml the growth of sections is very much inhibited. This effect usually takes up to 7 hours to become visible and is at a maximum in the 7 - 24 hour period of growth.

(iii) N.M.S.A. at concentrations of  $10^{-5}$  -  $5 \times 10^{-5}$  gm/ml causes a large stimulation of growth even in the first four hours. This stimulation persists over the period of 24 hours but after 24 hours it disappears.

(iv) There is a very small yet significant stimulation of about 5% by  $3 \times 10^{-6}$  and  $10^{-6}$  gm/ml P.C.I.B. An inhibition



effect appears within the first five hours at  $10^{-5}$  gm/ml.

(v) The amount of stimulation by F.N.B.A. at the optimum concentration of  $10^{-5}$  gm/ml is about 7%. At  $10^{-4}$  gm/ml the growth of sections is very much inhibited.

(b) The interaction of antiauxins with IAA

(i) An N.M.S.P. effect <sup>in</sup> the first seven hours of growth is not often observed but over the succeeding period from 7 - 24 hours there is at  $10^{-7}$  -  $10^{-5}$  gm/ml a well marked stimulation of growth. There is no interaction of IAA and N.M.S.P. in the first seven hours but this interaction is highly significant in the 7 - 24 hour period, due to a marked reduction in the stimulatory effect of N.M.S.P. by both concentrations of IAA.

(ii) P.C.I.B. in the first 5 hours of growth period causes a slight stimulation at  $3 \times 10^{-6}$  gm/ml. The highly significant interaction of P.C.I.B. and IAA in the first five hours is of the nature of a mutual antagonism between them such that growth in the mixture is lower than that in a solution of either of the compounds alone.

(iii) The interaction between IAA and C.N.B. is highly significant for the first three periods 0 - 4, 4 - 7 and 7 - 24 hours, but not in the last phase of growth. This interaction is also a mutual antagonism of stimulation of growth. It is seen in combinations of two concentrations of IAA with  $10^{-7}$  and  $10^{-6}$  gm/ml C.N.B.

(c) The mutual interaction of antiauxins

The interaction between N.M.S.A. and C.N.B. is highly significant over the periods of 0 - 4, 4 - 7 and 7 - 24 hours. There is a mutual antagonism of stimulation. It is very well marked in combinations of  $10^{-6}$  gm/ml N.M.S.A. with  $10^{-6}$ ,  $10^{-5}$  and  $10^{-4}$  gm/ml C.N.B.

Conclusion

Two major conclusions are to be drawn from the foregoing experimental results.

- 1) That auxin and its homologues are possibly functioning in identical ways in the same growth system. A critical survey of the action curves of all the compounds shows that the nature of the growth response is similar for all these compounds. The shapes of the ascending limbs of stimulation curves are very alike. The curves of the optimum responses have been superimposed. Even the shapes of the inhibition curves are also similar.
  - 2) The interaction patterns of mutual antagonism suggest a direct action of these compounds at the growth centres. Thus the idea of stimulation by the antagonism of an endogenous growth inhibitor may have to be abandoned.
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CHAPTER VII

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## SYMBOLS USED IN TABLES

The following letters are used for the various compounds appearing in the tables in the appendix:

I = B-indolyl-acetic acid

N = N.M.S.P. or N.M.S.A. (distinguishable in table readings)

P = P.C.I.B.

B = C.N.B.

For metals etc. the element symbol was used e.g.

Cu = Copper, Co = Cobalt, Mn = Manganese.

Suffixes represent the negative logarithm of concentration in gm/ml. for the auxin and antiauxins, e.g.

$I_{11} = 10^{-11}$  gm/ml. IAA and in molarity for the metals e.g.

$Co_5 = 10^{-5}$  M Cobalt chloride. E,  $E_2$  etc. refer to the separate experiment.



(1)

Age-effect  
Ratios of Corresponding Controls at 48 hours

Days after planting.

	1-day			2-day			3-day			4-day			5-day		
	E1	E2	E3	E1	E2	E3	E1	E2	E3	E1	E2	E3	E1	E2	E3
I <sub>0</sub>	97.62	103.12	104.05	96.76	100	104.56	99.79	100.21	102.56	100.27	95.32	100.90	96.07	106.33	103.61
	102.38	96.88	95.95	103.24	100	95.44	100.21	99.79	97.44	99.73	104.68	99.1	103.93	93.67	96.39
I <sub>11</sub>	102.38	101.68	100.67	103.28	96.67	104.51	102.75	96.42	104.90	100.27	103.49	100.90	111.73	102.53	96.39
	105.71	107.91	101.16	106.82	99.63	104.51	108.25	103.16	102.56	112.60	103.49	108.87	108.5	101.27	98.80
I <sub>8</sub>	89.52	92.32	89.11	85.02	85.42	84.02	81.18	82.11	82.98	92.76	89.32	81.25	85.45	84.56	86.75
	88.10	92.31	86.22	81.38	81.25	90.16	86.68	95.53	80.65	93.83	89.68	79.66	85.45	86.08	89.17

Age-effect  
Total Growth

	1-day			2-day			3-day			4-day			5-day		
	E1	E2	E3	E1	E2	E3	E1	E2	E3	E1	E2	E3	E1	E2	E3
I <sub>0</sub>	205	215	216	239	240	236	236	238	230	208	200	205	187	197	190
	210	202	204	245	240	236	237	237	229	205	185	200	186	179	205
I <sub>11</sub>	215	212	209	255	232	245	243	229	235	212	205	200	197	190	205
	222	225	210	264	229.5	248	256	245	230	215	200	206	200	190	190
I <sub>10</sub>	215	207	215	240	228	240	227	225	227	214	180	207	190	180	182
	208	205	205	235	217	245	235	228	235	202	183	189	175	190	182
I <sub>8</sub>	188	192.5	179	210	205	210	192	195	188	175	167	170	173	164	157
	185	192.5	175	201	195	215	205	205	180	176	170	175	170	165	153

(2)

Effect of Light

Ratios to light control

Intensity of light = 80 foot candle.

Conc. IAA	Light			Dark		
	E1	E2	E3	E1	E2	E3
I <sub>0</sub>	98.4	100	100.2	97.3	111.8	109.0
	101.6	100	99.8	93.9	110.8	97.8
I <sub>11</sub>	99.3	109.3	104.2	98.4	118.2	112.5
	97.1	100	107.6	102.0	120.7	112.0
I <sub>10</sub>	112.9	108.4	109.0	93.4	101.0	103.2
	97.1	105.9	118.0	90.3	102.0	95.8
I <sub>9</sub>	106.1	97.0	106.3	89.2	94.6	97.8
	94.8	106.5	98.8	85.8	108.8	97.7
I <sub>8</sub>	90.3	90.1	95.3	81.3	82.3	80.2
	88.0	88.7	93.9	92.5	81.3	75.8
I <sub>7</sub>	73.4	73.9	72.9	81.3	80.3	65.0
	72.2	72.4	73.3	80.6	81.3	65.0

Effect of Manganese in Light and Dark

Ratios to light control

Intensity of light = 120 foot candle.

	Light						Dark					
	E1		E2		E3		E1		E2		E3	
	O	Mn5	O	Mn5	O	Mn5	O	Mn5	O	Mn5	O	Mn5
I <sub>0</sub>	101.9	102.0	104.8	101.7	99.7	103.3	97.7	105.9	97.4	97.7	101.5	100.0
	98.1	106.7	95.2	101.8	100.2	102.0	101.1	107.2	98.3	97.4	102.1	92.7
I <sub>11</sub>	103.3	101.6	109.1	103.9	103.3	100.2	106.9	100.5	97.4	95.3	104.6	103.3
	101.0	104.6	103.9	105.4	106.6	98.9	105.8	101.1	100.3	98.8	105.0	101.1
I <sub>10</sub>	109.0	102.4	111.3	109.1	105.0	107.6	98.1	101.4	100.3	96.2	95.4	96.3
	103.7	98.6	106.5	104.8	111.2	106.4	96.8	98.9	98.8	93.2	96.7	97.1

AA.

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Concentration-response curve of calcium

Ratios of the corresponding controls.

		Concentrations of Calcium (Molar)						
		0	10 <sup>-8</sup>	10 <sup>-7</sup>	10 <sup>-6</sup>	10 <sup>-5</sup>	10 <sup>-4</sup>	10 <sup>-3</sup>
E <sub>1</sub>	100.	90.5	88.8	104.5	98.9	104.5	73.0	
	100	93.8	95.8	104.0	98.9	107.0	70.0	
E <sub>2</sub>	101.3	94.7	98.2	94.7	90.0	92.3	75.3	
	98.7	93.3	94.7	95.1	90.7	94.5	74.4	
E <sub>3</sub>	102.2	100.8	94.6	93.3	100.0	96.6	74.0	
	97.8	100.0	100.8	93.0	100.8	95.1	80.0	

Interaction of Calcium and IAA

Ratios to corresponding controls (at 48 hours)

Concentrations in Molar.

		0	C <sub>a8</sub>	0	C <sub>a7</sub>	0	C <sub>a6</sub>	0	C <sub>a5</sub>	0	C <sub>a4</sub>
I <sub>0</sub>	E <sub>1</sub>	96.8	106.2	97.6	89.8	103.65	94.52	102.7	111.8	98.7	92.3
		103.2	101.7	102.8	104.9	93.67	99.09	97.3	102.2	101.3	106.5.
						102.68	103.65				
	E <sub>2</sub>	91.2	90.3	100.2	104.9	101.65	99.98	101.7	105.7	98.1	91.3
		108.8	93.9	99.8	103.6	95.85	95.85	99.3	97.3	101.9	76.9
						102.54	96.29				
	E <sub>3</sub>	X	X	X	X	101.0	99.0	X	X	X	X
						100.	99.0				
						99.0	95.0				
I <sub>11</sub>	E <sub>1</sub>	119.6	96.3	95.1	105.4	109.13	105.02	122.0	107.5	105.6	101.9
		110.2	108.7	104.4	97.6	105.94	97.76	120.1	112.9	104.0	104.0
						102.74	97.72				
	E <sub>2</sub>	98.6	97.2	109.7	113.1	111.9	102.1	119.4	122.9	98.6	100.5
		103.2	108.8	105.5	105.9	106.1	102.1	118.4	122.9	90.4	105.3
						104.77	104.99				
	E <sub>3</sub>	X	X	X	X	108.0	102.5				
						106.25	99.0	X	X	X	X
						103.0	105.0				
I <sub>8</sub>	E <sub>1</sub>	86.8	89.8	82.4	93.7	82.19	87.67	102.1	100.8	88.0	89.6
		89.3	97.8	86.8	88.8	83.10	86.70	96.8	87.6	93.3	96.0
						85.39	89.04				
	E <sub>2</sub>	80.1	85.6	83.1	96.4	84.71	86.94	95.3	93.4	84.1	78.8
		86.6	93.9	79.8	102.1	93.62	90.28	95.8	85.9	76.9	84.1
						94.07	88.72				
	E <sub>3</sub>	X	X	X	X	85.75	89.5				
						84.0	85.5	X	X	X	X
						86.25	92.5				



### Concentration-response curve of Manganese

Ratios to corresponding control at 48 hours

Conc. (molar) manganese							
	0	$10^{-8}$	$10^{-7}$	$10^{-6}$	$10^{-5}$	$10^{-4}$	$10^{-3}$
E <sub>1</sub>	100	104	102	106	109	104	47
	100	102	101	105	105	106	47
E <sub>2</sub>	102	100	101	104	103	103	48
	98	97	103	103	106	105	49

## IAA-Manganese Interactions

Ratios to corresponding controls at 48 hours

Conc. (molar) manganese											
		0	10 <sup>-8</sup>	0	10 <sup>-7</sup>	0	10 <sup>-6</sup>	0	10 <sup>-5</sup>	0	10 <sup>-4</sup>
I <sub>0</sub>	E <sub>1</sub>	101.9	103.7	101.5	97.3	98.1	108.6	97.16	109.0	106.0	92.6
		98.1	123.1	98.5	98.5	101.9	102.3	101.07	109.0	94.0	95.4
								101.87	110.38		
	E <sub>2</sub>	96.8	114.4	96.9	111.8	100	91.8	98.06	109.35	100	110.2
		103.2	112.1	103.1	107.5	100	96.6	101.67	103.95	100	115.8
								100 32	101 67		
I <sub>111</sub>	E <sub>1</sub>	106.5	118.1	100.2	111.6	97.3	100.2	101.86	101.07	120.0	119.4
		103.2	122.7	98.5	109.9	91.9	106.9	103.05	103.05	118.0	118.5
								103 05	104.24		
	E <sub>2</sub>	106.1	96.8	99.1	101.8	89.2	99.2	102.12	106.19	112.4	115.2
		109.5	105.1	102.6	95.2	89.2	104.4	103.03	102.58	111.2	114.1
								101 67	104.61		
I <sub>8</sub>	E <sub>1</sub>	83.3	93.5	83.8	101.5	73.5	88.9	74.9	82.4	89.9	97.2
		101.9	95.8	75.4	92.2	95.2	81.0	72.4	82.7	101.1	96.8
	E <sub>2</sub>	92.9	90.0	90.1	85.9	77.9	93.6	90.3	82.3	97.2	102.8
		95.9	97.0	93.0	74.6	84.9	87.0	81.9	76.8	100.0	104.5

Concentration-response curve of Zinc

Ratios to corresponding  
controls.

		Conc. (molar) Zinc							
		0	10 <sup>-10</sup>	10 <sup>-9</sup>	10 <sup>-8</sup>	10 <sup>-7</sup>	10 <sup>-6</sup>	10 <sup>-5</sup>	10 <sup>-4</sup>
E <sub>1</sub>		100.3	85.5	82.7	90.0	96.0	93.8	97.6	88.0
		99.7	83.5	80.0	81.6	96.0	88.0	95.7	82.0
E <sub>2</sub>		104.4	95.0	103.0	104.4	99.5	94.5	89.5	78.1
		95.6	89.8	97.0	102.0	106.0	106.0	94.5	73.6
E <sub>3</sub>		100	94.8	85.2	96.2	98.0	96.3	93.8	70.0
		100	92.0	91.0	97.0	99.2	98.8	93.8	69.1

IAA-Zinc-Interaction

Ratios to corresponding control.

		0	$10^{-7}$	0	$10^{-6}$	0	$10^{-5}$	0	$10^{-4}$
$I_0$	$E_1$	98.8	98.8	101.55	97.93	102.74	100.1	107.44	72.09
		101.2	99.75	98.45	106.22	98.63	92.21	100	76.74
	$E_2$					98.63	105.02	92.56	71.16
		101.46	102.43	96.71	94.95	95.23	102.74	100.85	76.43
$I_{11}$	$E_1$	99.75	103.61	103.63	113.98	95.43	102.74	102.3	72.09
		103.61	97.56	104.65	99.45	100.	100.46	102.3	67.44
	$E_2$					100.4	95.43	104.68	69.77
		106.34	103.41	103.74	98.02	97.27	99.59	97.66	73.04
$E_8$	$E_1$	93.98	88.55	92.31	95.85	92.31	85.13	80.2	80.7
		89.16	81.93	81.32	91.19	89.74	85.13	80.2	77.71
	$E_2$					89.74	85.23	80.0	70.7
		87.80	80.49	92.35	83.52	80.54	89.93	80.85	60.
	$E_2$	80.98	81.95	81.32	87.91	80.98	85.07	79.57	62.13
						80.98	85.01	80.85	62.13



Concentration-response curve of Boron

Ratios to corresponding controls

		Conc. (molar) Boron							
		0	10 <sup>-9</sup>	10 <sup>-8</sup>	10 <sup>-7</sup>	10 <sup>-6</sup>	10 <sup>-5</sup>	10 <sup>-4</sup>	10 <sup>-3</sup>
E <sub>1</sub>		95.9	93.1	91.2	95.0	95.5	95.2	90.7	84.1
		104.1	101.6	87.4	85.1	93.1	94.5	90.7	76.6
E <sub>2</sub>		98.7	98.5	98.6	99.7	94.8	103.2	95.7	87.3
		101.3	99.2	101.3	105.7	96.8	97.0	97.7	87.3

IAA-Boron Interaction

Ratios to corresponding controls

Conc. (molar) Boron.

		0	10 <sup>-6</sup>	0	10 <sup>-5</sup>	0	10 <sup>-4</sup>	0	10 <sup>-3</sup>
I <sub>0</sub>	E <sub>1</sub>	96.5	105.0	99.76	96.96	100.4	98.39	99.3	91.31
		103.5	101.5	100.26	99.29	100.8	99.6	100.98	90.47
	E <sub>2</sub>	101.92	104.93	100	100.5	99.23	96.98	100.22	93.53
		98.08	101.86	100	102.01	99.23	100.36	97.15	98.51
	E <sub>3</sub>					101.49	97.88	101.58	99.41
						93.03	102.44		
I <sub>11</sub>	E <sub>1</sub>	105	107.5	96.49	101.41	95.56	95.16	98.91	90.47
		110.5	109.0	97.89	99.76	101.81	103.63	101.01	90.47
	E <sub>2</sub>	103.64	101.07	113.07	113.07	98.33	94.72	107.55	93.53
		98.22	106.2	102.51	109.05	103.74	94.72	110.71	96.24
	E <sub>3</sub>					97.88	94.72	106.19	98.06
						98.26	94.08		
I <sub>8</sub>	E <sub>1</sub>	95.0	87.5	86.65	81.97	83.58	89.55	84.76	76.2
		90.0	95.0	89.46	79.63	86.51	88.06	82.86	83.33
	E <sub>2</sub>	91.65	90.36	97.99	89.45	89.66	95.17	96.67	86.79
		85.65	85.65	95.48	93.97	92.41	95.17	91.51	91.98
	E <sub>3</sub>					85.23	88.25		
						84.69	84.40		

Concentration-response curve of Cobalt

Ratios to corresponding controls at 48 hours.

*Conc. (molar) Cobalt.*

	0	10 <sup>-8</sup>	10 <sup>-7</sup>	10 <sup>-6</sup>	10 <sup>-5</sup>	10 <sup>-4</sup>
E <sub>1</sub>	100.6	91.2	96.3	106.6	115.6	84.6
	105.6	100.6	94.1	106.0	113.6	86.9
	93.8	104.9	100.2	104.9	115.6	82.0
E <sub>2</sub>	104.3	94.8	90.5	107.7	110.9	84.9
	98.7	97.0	97.0	102.9	116.3	80.6
	97.0	94.3	87.0	104.5	113.4	90.5

IAA-Cobalt Interaction

Ratios to corresponding control

*Conc. (molar) Cobalt.*

		0	10 <sup>-7</sup>	0	10 <sup>-6</sup>	0	10 <sup>-5</sup>	0	10 <sup>-4</sup>
I <sub>0</sub>	E <sub>1</sub>	100	102.8	100	97.87	98.49	109.94	100.51	94.53
		100	97.2	100	103.83	103.12	112.69	98.37	97.52
						98.49	112.0	101.12	89.82
	E <sub>2</sub>	99.79	100.21	102.73	104.18	101.4	122.09	101.21	95.14
		100.21	102.35	93.20	101.94	99.43	119.91	101.21	91.5
						99.17	122.75	97.58	92.3
I <sub>11</sub>	E <sub>1</sub>	108.0	98.80	105.32	106.81	100.78	112.23	104.79	94.1
		103.0	98.0	112.92	111.7	105.13	112.23	106.93	100.08
						100.78	114.06	100.94	96.24
	E <sub>2</sub>	95.95	98.5	94.1	100.82	115.55	118.82	97.57	89.85
		95.52	100.0	103.06	104.0	102.47	108.57	100.81	87.04
						104.65	113.37	101.21	95.14
I <sub>8</sub>	E <sub>1</sub>	70.40	88.0	83.62	100	88.41	97.57	90.68	92.39
		75.80	88.0	87.66	102.13	85.66	100.78	87.68	92.39
						85.66	100.78	94.1	91.96
	E <sub>2</sub>	91.68	100.21	81.4	100.82	91.13	104.65	87.04	91.09
		86.14	90.20	89.62	103.06	95.93	105.52	83.81	88.26
						95.06	107.7	85.02	91.09



Cobalt-IAA Interaction

Growth in % per hour.

Conc  $2\text{m/ml}$  I.A.A.

		0		-11 10		-10 10	
		Co(0)	Co5	Co(0)	Co5	Co	Co <sup>5</sup>
0 ↓ 4	E <sub>1</sub>	5.0	8.2	7.5	7.2	7.0	6.5
		5.0	8.7	7.5	7.2	6.0	7.0
	E <sub>2</sub>	6.0	8.2	8.7	7.2	8.5	8.0
		6.5	8.2	8.7	7.7	8.0	8.0
	E <sub>3</sub>	6.5	7.2	7.2	7.5	8.5	7.2
		6.0	7.5	8.5	7.2	8.0	7.0
4 ↓ 7	E <sub>1</sub>	7.6	9.3	8.0	8.0	8.3	9.3
		7.3	9.0	8.0	7.9	8.6	8.3
	E <sub>2</sub>	6.3	8.0	7.0	6.0	6.3	8.0
		6.3	7.3	6.6	7.6	7.3	7.6
	E <sub>3</sub>	7.4	9.2	8.0	9.5	7.2	8.0
		7.2	9.6	7.0	9.5	7.6	8.1
7 ↓ 24	E <sub>1</sub>	7.2	8.7	7.7	8.8	7.4	8.4
		7.3	9.0	7.8	9.0	7.7	9.0
	E <sub>2</sub>	7.0	8.0	8.0	7.8	7.7	8.0
		7.8	8.0	8.0	7.6	7.6	7.7
	E <sub>3</sub>	9.1	9.0	9.3	9.0	9.3	9.0
		8.6	9.3	9.1	8.8	9.2	9.0
24 ↓ 48	E <sub>1</sub>	1.2	1.5	1.3	.9	1.2	.8
		1.2	1.2	1.2	.8	1.3	.8
	E <sub>2</sub>	1.1	1.9	1.1	1.5	1.4	1.5
		1.0	2.0	1.3	1.5	1.3	1.5
	E <sub>3</sub>	1.5	2.0	1.8	2.0	1.7	1.7
		1.5	2.2	1.7	2.2	1.5	2.2

Concentration-response curve of Copper

Ratios to corresponding  
controls

		Conc. (molar) Copper				
		0	$10^{-9}$	$10^{-8}$	$10^{-7}$	$10^{-6}$
$E_1$		98.5	100	97.5	96.0	93.0
		101.5	92.5	97.2	92.5	91.0
$E_2$		99.2	98.2	96.5	94.3	92.7
		100.8	96.3	97.9	96.2	91.5

IAA-Copper Interaction

Ratios to corresponding controls

		Conc. (molar) Copper					
		0	$10^{-8}$	0	$10^{-7}$	0	$10^{-6}$
$I_0$	$E_1$	100	91.7	96.7	98.7	99.4	83.6
		100	95.8	103.3	100.8	100.6	84.4
	$E_2$	96.8	98.6	99.6	96.8	94.2	100.5
		103.2	90.4	100.4	90.5	105.8	92.6
$I_{11}$	$E_1$	100.0	95.8	109.8	103.3	101.8	108.
		97.9	94.6	108.3	103.3	104.9	102.6
	$E_2$	111.9	105.0	104.2	105.6	116.4	97.9
		107.3	101.8	100.2	103.5	124.3	100.5
$I_8$	$E_1$	77.1	77.1	92.2	97.3	88.8	80.4
		80.3	75.0	90.7	87.7	76.9	84.7
	$E_2$	87.7	79.0	85.6	73.3	95.2	75.0
		83.6	78.5	74.8	77.3	89.9	73.0



(11)  
Concentration-response curves of the action of  
B-indolylacetic acid (IAA)

Growth in % / hour

Conc. of IAA $\mu\text{m}$ l											
		I 0	I 14	I 13	I 12	I 11	I 10	I 9	I 8	I 7	
0	E 1	7.6	7.9	7.5	8.1	9.0					
		5.8	7.5	7.9	7.9	10.3					
	E 2	6.7	8.0	9.4	10.0	9.0					
		6.7	7.4	7.6	9.6	10.0					
	E 3	6.8	7.1	8.5	8.0	10.4					
4		6.6	6.3	7.2	7.1	10.4					
	E 4	6.9					8.10	8.3	3.7	2.5	
		6.5					8.7	8.3	3.9	2.7	
	E 5	6.6					7.6	7.2	3.3	2.3	
		6.8					8.0	6.8	3.1	2.1	
4	E 1	7.2	7.0	7.5	7.2	9.3					
		8.3	7.2	7.0	7.2	9.6					
	E 2	7.0	7.0	7.7	8.3	11.1					
		8.5	7.0	8.5	8.1	11.6					
	E 3	8.5	8.5	7.0	8.5	7.1					
7		7.0	7.7	8.5	7.7	7.4					
	E 4	8.2					9.2	8.9	5.0	3.6	
		7.3					8.9	7.3	5.3	3.2	
	E 5	7.9					7.6	6.7	6.0	2.6	
		7.6					7.3	7.0	5.0	2.9	
7	E 1	8.0	7.8	8.0	8.3	8.0					
		8.1	7.8	7.6	8.3	7.8					
	E 2	8.0	7.7	8.2	8.4	8.1					
		8.1	7.4	7.1	7.7	8.1					
	E 3	8.3	8.0	8.5	8.5	8.5					
24		7.8	7.7	8.0	8.0	8.9					
	E 4	8.4					7.8	8.0	6.0	4.6	
							8.0	7.5	6.0	4.4	
	E 5	8.1					8.2	8.1	5.8	4.7	
		8.0							6.0	5.0	
24	E 1	1.3	1.3	1.3	1.3	1.7					
		1.4	1.5	1.4	1.3	1.7					
	E 2	1.4	1.5	1.6	1.4	1.4					
		1.3	1.3	1.4	1.6	1.4					
	E 3	1.3	1.1	1.2	1.1	1.1					
48		1.3	1.1	1.2	1.4	1.1					
	E 4	1.4					1.4	1.1	1.1	.9	
		1.3					1.5	1.5	1.1	.9	
	E 5	1.3					1.1	1.2	1.3	1.0	
		1.4					1.4	1.3	1.2	1.0	

Concentration-response curves of the action  
of IAA

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Table of average

Concentration of IAA <i>gm/ml</i>									
	C	I <sub>14</sub>	I <sub>13</sub>	I <sub>12</sub>	I <sub>11</sub>	I <sub>10</sub>	I <sub>9</sub>	I <sub>8</sub>	I <sub>7</sub>
0	n=10	n=6	→						
4	6.70	7.37	8.02	8.45	9.85	8.10	7.65	3.5	2.4
4 7	7.75	7.40	7.7	8.0	9.35	8.25	7.47	5.32	3.07
7 24	8.05	7.73	7.90	8.20	8.23	8.05	7.85	5.95	4.67
24 48	1.34	1.27	1.30	1.35	1.45	1.35	1.28	1.18	.95



curve  
Concentration-growth response/ of N.M.S.P.

Ratios to corresponding controls at 48 hours.

		Conc. $\frac{\text{gm}}{\text{ml}}$ N.M.S.P.									
		0	$10^{-10}$	$10^{-9}$	$10^{-8}$	$10^{-7}$	$10^{-6}$	$10^{-5}$	$10^{-4}$	$5 \times 10^{-4}$	$10^{-3}$
E <sub>1</sub>		99.3	100.9	101.2	100.0	110.7	119.8	136.0	105.6	98.6	81.3
		100.7	100.0	102.3	103.9	104.0	120.6	134.0	109.1	95.3	78.1
E <sub>2</sub>		99.1	96.4	95.6	102.2	108.8	121.8	130.4	107.7	96.7	71.1
		100.9	98.2	97.8	104.2	106.7	118.9	139.6	109.6	92.2	70.2
E <sub>3</sub>		99.8	101.1	98.9	101.1	109.7	107.5	122.6	110.3	101.1	72.2
		100.2	103.2	104.5	101.9	107.5	111.8	126.5	110.3	96.8	73.9

Concentration-growth response curve of N.M.S.A.

Ratios to corresponding controls at 48 hours.

		Conc. $\mu\text{g/ml}$ N.M.S.A.					
	0	$10^{-8}$	$10^{-7}$	$10^{-6}$	$10^{-5}$	$5 \times 10^{-5}$	$10^{-4}$
E <sub>1</sub>	100.4	106.2	104.9	108.9	125.9	127	122.7
	99.2	94.6	100.9	108.5	127.2	129.4	116.1
	100.4	100.4	94.6	102.6	127.2	128.5	122.3
E <sub>2</sub>	97.8	93.4	93.3	106.6	120.3	117.4	117.4
	102.2	102.2	95.4	110.	121.0	116.4	115.9
E <sub>3</sub>	100	100.3	100.0	104.0	124.4	121.3	112.4
	100	100.0	100.9	105.8	126.0	117.8	116.4

Concentration-growth response curve of N.M.S.A.

Growth in % per hour

		Conc. $\mu\text{g/ml}$		N.M.S.A.				
		0	$10^{-8}$	$10^{-7}$	$10^{-6}$	$10^{-5}$	$5 \times 10^{-5}$	$10^{-4}$
0 ↓ 4	E <sub>1</sub>	4.5	4.7	4.7	5.2	7.25	7.0	5.75
		4.7	4.6	4.5	5.6	7.25	7.2	5.5
		4.7	4.7	4.9	5.9	7.75	7.4	6.0
	E <sub>2</sub>	4.25	3.75	4.7	5.75	7.25	7.2	4.5
		4.7	5.5	5.2	4.25	7.5	7.0	5.25
	E <sub>3</sub>	4.7	5.2	5.2	5.75	6.5	6.0	5.5
		5.2	5.0	5.5	6.0	7.75	6.2	5.75
4 ↓ 7	E <sub>1</sub>	8	9.0	7.5	8.7	10.7	11.3	10.7
		8	7.5	8.3	7.5	11.0	11.3	11.3
		8.3	8.3	8.2	7.2	10.0	10.8	10.0
	E <sub>2</sub>	6.6	6.0	7.0	7.7	8.7	9.0	10.0
		6.0	5.7	7.1	8.3	9.0	9.3	9.0
	E <sub>3</sub>	7.3	7.0	7.3	7.0	9.7	9.3	7.7
		7.0	7.6	6.3	7.0	8.0	8.6	7.7
7 ↓ 24	E <sub>1</sub>	8.7	8.6	8.8	9.6	11.7	11.3	11.7
		8.3	7.9	7.8	9.2	11.6	12.1	10.8
		8.6	8.3	7.4	8.5	11.4	11.2	10.8
	E <sub>2</sub>	8.3	8.3	8.0	8.5	9.7	9.5	10.1
		8.6	8.2	8.0	9.2	9.8	9.1	10.2
	E <sub>3</sub>	8.7	8.6	8.3	8.7	11.2	11.0	10.2
		8.1	8.4	8.6	9.3	11.2	11.4	10.8
24 ↓ 48	E <sub>1</sub>	1.4	1.9	1.8	1.4	.9	1.2	.8
		1.5	1.5	2.0	1.7	1.0	.8	.8
		1.4	1.6		1.6	1.2	1.4	1.5
	E <sub>2</sub>	.9	.7	.6	1.1	1.1	.9	.8
		1.1	1.2	.8	.9	1.0	1.0	.7
	E <sub>3</sub>	1.5	1.5	1.6	1.7	1.4	1.1	1.3
		1.8	1.6	1.6	1.4	1.6	1.1	1.4



Concentration growth response curve of P.C.I.B.

Ratios to corresponding controls at 48 hours.

	Conc. gm/ml						P.C.I.B.
	0	$10^{-8}$	$10^{-7}$	$10^{-6}$	$10^{-5}$	$10^{-4}$	
E <sub>1</sub>	101.7	95.9	97.4	105.3	70.8	very toxic	
	100.9	95.4	100.3	103.0	71.7		
	97.4	101.4	104.4	100.3	69.9		
E <sub>2</sub>	98.2	95.9	93.6	102.0	78.1	x	
	102.3	95.4	97.3	102.3	81.0		
	99.5	100.4	95.9	104.3	81.0		
E <sub>3</sub>	103.3	92.3	94.9	107.7	80.4	x	
	96.7	94.1	97.2	107.9	79.6		
	100.0	93.2	96.1	106.5	80.0		

Concentration-response curve of C.N.B.

Ratios to corresponding controls at 48 hours

		Conc.	C.N.B.					
		0	$10^{-8}$	$10^{-7}$	$10^{-6}$	$10^{-5}$	$10^{-4}$	$5 \times 10^{-4}$
E <sub>1</sub>	99.2		103.5	104.8	106.6	115.4	95.6	76.7
	100.8		99.5	104.3	101.3	112.0	93.4	76.3
E <sub>2</sub>	102.1		102.1	102.1	107.3	114.0	94.1	75.2
	97.7		104.5	100.	105.9	113.5	93.5	75.4
E <sub>3</sub>	100.6		100.6	94.6	102.3	114.0	85.1	70.9
	99.4		98.9	96.3	101.5	114.0	89.0	75.2

Concentration-response curve of C.N.B.

Growth in % per hour

		Conc.	C.N.B.					
		0	$10^{-8}$	$10^{-7}$	$10^{-6}$	$10^{-5}$	$10^{-4}$	$5 \times 10^{-4}$
0	E <sub>1</sub>	4.2	5.0	3.7	6.0	4.7	5.5	4.0
		5.0	5.5	5.0	5.0	5.5	4.0	3.0
	E <sub>2</sub>	4.7	4.2	4.5	5.5	7.7	6.2	3.7
		4.2	5.5	5.7	7.0	6.5	6.5	4.2
4	E <sub>2</sub>	5.0	5.5	4.0	5.2	6.7	4.2	4.0
		5.0	5.0	4.2	6.2	6.5	5.5	4.0
4	E <sub>1</sub>	4.6	4.0	5.6	5.0	6.3	6.3	3.3
		5.6	5.3	4.0	5.0	5.3	5.3	3.3
	E <sub>2</sub>	4.3	5.3	5.3	4.3	5.3	4.0	3.3
		5.6	4.0	3.5	5.0	5.0	3.8	2.6
7	E <sub>3</sub>	4.6	4.0	5.6	5.0	5.6	5.5	5.0
		4.3	4.0	5.0	4.8	5.6	5.0	4.6
7	E <sub>1</sub>	8.8	8.4	8.4	8.9	10.4	9.2	8.0
		8.4	8.0	8.3	8.5	9.8	9.4	7.9
	E <sub>2</sub>	9.0	8.9	8.6	9.0	10.0	8.6	8.4
		8.0	8.7	8.7	9.0	10.2	8.5	8.0
24	E <sub>3</sub>	9.0	9.0	8.9	8.8	11.0	8.6	7.2
		8.6	9.0	9.0	9.1	11.2	9.6	7.6
24	E <sub>1</sub>	1.9	2.2	2.1	2.2	2.0	.9	.6
		2.0	2.0	2.2	2.2	2.1	.8	.7
	E <sub>2</sub>	1.2	1.3	1.4	1.6	1.3	.7	.6
		1.4	1.5	1.2	1.5	1.3	.7	.5
48	E <sub>3</sub>	1.7	2.0	1.8	2.2	1.3	.6	.4
		2.2	1.5	1.7	1.8	1.3	.5	.7



Concentration-growth response curve of F.N.B.A.

Ratios to corresponding controls at (48) hours.

	Conc. $\mu\text{m/ml}$ F.N.B.A.					
	0	$10^{-8}$	$10^{-7}$	$10^{-6}$	$10^{-5}$	$10^{-4}$
E <sub>1</sub>	99	99.0	101.1	108.0	108.5	94.2
	101	94.2	100.5	106.7	109.4	99.0
E <sub>2</sub>	103.0	100.6	99.3	104.0	108.5	99.0
	97	103.5	101.6	104.8	106.2	102.0
E <sub>3</sub>	96.6	102.3	102.3	106.9	107.0	102.3
	103.4	96.2	102.3	102.3	106.5	101.4

## Interaction between N.M.S.P. and IAA

Growth (Fresh Weight) percentage per hour (rate). Original table.

		I <sub>0</sub>								I <sub>11</sub>								I <sub>10</sub>							
		No	N <sub>8</sub>	No	N <sub>7</sub>	No	N <sub>6</sub>	No	N <sub>5</sub>	No	N <sub>8</sub>	No	N <sub>7</sub>	No	N <sub>6</sub>	No	N <sub>5</sub>	No	N <sub>8</sub>	No	N <sub>7</sub>	No	N <sub>6</sub>	No	N <sub>5</sub>
0	E1	7.2	8.2	7.0	7.8	6.5	6.5	8.8	6.5	8.5	8.8	7.5	7.5	7.8	6.2	9.8	8.3	7.2	7.2	7.2	5.9	6.5	7.5	7.3	7.8
		6.0	6.2	7.0	6.9	6.0	6.2	8.0	7.5	8.0	8.0	7.0	7.5	7.0	6.9	8.0	8.6	7.8	7.2	7.2	7.5	6.0	6.9	8.6	8.0
	E2	5.5	5.8	6.8	5.5	8.5	6.8	7.2	7.0	6.5	4.8	7.5	7.5	8.0	9.2	8.0	5.5	4.8	6.0	6.5	7.2	6.8	8.2	5.3	5.0
		5.5	6.5	7.0	6.5	7.5	7.2	6.8	6.0	5.5	5.2	9.5	7.0	9.0	8.2	7.0	5.0	5.5	5.5	7.2	7.5	7.2	6.5	5.0	5.5
4	E3	6.0	6.0	6.0	7.0	8.5	7.8	5.7	6.5	7.2	7.0	9.5	8.5	8.8	8.2	7.0	5.7	6.8	6.2	9.0	8.0	8.2	7.2	5.0	7.0
		6.8	6.0	7.0	5.5	7.8	7.5	5.0	5.0	7.8	7.0	8.2	7.0	9.0	9.0	7.8	7.0	6.5	7.0	7.5	8.0	6.0	6.5	5.0	6.0
	E1	5.7	7.6	6.3	6.3	7.3	7.7	6.3	7.0	7.0	7.0	6.7	8.0	7.6	7.3	8.0	7.7	6.0	6.0	6.0	6.3	7.0	5.0	7.7	7.3
		6.7	7.0	6.3	6.7	5.7	7.7	6.7	8.0	5.7	5.0	6.0	6.0	6.0	5.7	6.3	7.0	5.7	5.0	5.0	6.0	6.0	7.0	6.3	6.8
7	E2	6.0	7.3	6.0	6.6	6.3	6.0	6.5	5.3	6.2	6.3	7.0	5.7	6.7	5.3	6.0	6.3	5.7	7.0	5.0	7.0	6.3	5.7	4.7	6.3
		5.3	7.3	6.3	7.3	8.6	5.0	5.3	5.0	6.3	6.7	6.7	5.7	8.6	5.0	6.3	5.6	8.3	5.7	5.3	5.3	5.3	5.3	5.3	5.9
	E3	7.0	7.3	8.6	8.0	6.7	7.7	6.7	6.0	8.3	6.7	10.3	8.0	7.0	8.0	8.3	7.0	5.7	7.7	8.3	9.0	6.3	6.7	8.0	5.3
		6.7	7.3	8.3	8.0	8.3	7.0	5.7	6.0	9.3	8.7	10.0	8.6	8.6	6.3	6.0	6.7	6.3	7.3	9.3	8.3	5.0	8.0	8.0	5.3
24	E1	10.0	8.4	8.7	9.0	8.4	9.7	8.6	12.5	9.3	8.4	8.1	8.2	7.8	9.0	8.3	11.1	8.6	8.7	7.8	8.3	8.5	9.3	7.8	10.6
		9.0	8.7	8.0	8.7	7.5	10.3	8.1	11.2	9.6	8.9	8.3	8.1	8.5	10.0	8.5	10.0	8.2	8.9	8.3	7.8	8.7	9.8	8.0	10.5
	E2	8.4	9.0	9.7	10.7	8.9	10.8	8.3	11.4	8.5	8.2	8.9	9.6	8.4	10.5	8.2	9.7	7.9	8.4	10.0	9.6	8.2	9.5	8.1	9.5
		8.5	8.3	9.3	10.2	8.3	11.5	7.8	11.8	9.0	8.0	9.5	9.4	8.7	9.4	9.5	9.8	7.2	8.6	9.7	9.7	9.0	9.9	8.1	10.5
48	E3	9.0	9.2	8.2	9.3	7.8	10.3	7.0	10.9	8.6	9.0	7.8	8.4	8.8	8.3	8.9	10.4	8.8	9.0	9.0	9.0	8.4	9.7	7.3	10.2
		9.3	9.5	8.5	9.5	8.3	10.4	7.3	10.7	8.4	8.0	8.4	8.4	8.6	9.4	8.3	9.8	8.2	8.0	8.0	8.0	8.7	9.3	8.4	9.9
	E1	1.1	1.4	1.1	1.5	1.3	1.4	1.0	2.3	1.5	1.5	1.7	1.5	1.9	1.8	1.3	1.6	2.0	1.5	1.6	1.5	1.4	1.8	1.3	1.3
		1.6	1.7	1.5	1.6	1.8	1.5	1.3	1.7	1.6	1.6	1.9	1.3	1.4	1.2	1.0	1.5	1.5	1.8	1.3	1.8	1.5	1.5	1.6	1.4
96	E2	.9	1.6	1.7	2.0	.9	2.3	1.1	1.9	1.4	1.5	2.0	1.1	1.0	1.9	1.0	1.9	1.5	1.4	1.8	1.4	1.0	1.4	1.2	1.6
		1.1	1.3	1.9	1.5	1.0	1.9	1.3	1.8	1.5	1.6	1.4	1.9	1.0	1.5	1.9	1.5	1.0	1.2	1.7	1.5	1.5	1.4	1.3	1.9
	E3	1.0	1.1	1.6	1.3	1.0	1.4	1.6	1.8	1.3	1.5	1.5	1.3	1.5	1.8	1.4	1.5	1.5	.9	1.5	1.1	1.5	1.0	1.6	1.5
		1.2	1.2	1.5	1.7	1.0	1.4	1.5	1.9	1.2	1.3	1.6	1.4	1.6	1.1	1.9	1.5	1.4	.8	1.3	1.4	1.2	1.3	1.6	1.1



N.M.S.P.-pretreatment for first 6 hours

Growth in % per hour

		Conc. $\mu\text{m/l}$			N.M.S.P.		
		0			$10^{-5}$		
		E <sub>1</sub>	E <sub>2</sub>	E <sub>3</sub>	E <sub>1</sub>	E <sub>2</sub>	E <sub>3</sub>
0	↓	7.5	6.5	5.8	9.5	8.3	8.7
		6.7	6.3	5.6	9.3	9.0	8.1
		6.7	6.8	5.8	9.0	9.5	7.1
		7.7	6.8	5.3	8.3	9.0	7.5
		7.1	6.1	5.0	7.7	8.3	7.0
		7.5	6.7	5.8	8.1	8.3	6.8
6	↓	7.3	6.7	5.6	8.5	8.7	6.0
		7.7	6.8	5.8	7.3	8.3	7.0

Subsequent to N.M.S.P.-pretreatment, washed  
and IAA treated to washed sections

		Conc. $\mu\text{m/l}$ IAA							
		0		$10^{-11}$		$10^{-10}$		$10^{-8}$	
		N <sub>0</sub>	N <sub>5p</sub>	N <sub>0</sub>	N <sub>5p</sub>	N <sub>0</sub>	N <sub>5p</sub>	N <sub>0</sub>	N <sub>5p</sub>
0	E <sub>1</sub>	7.0	6.3	7.4	6.5	7.3	5.8	4.5	4.7
		7.4	6.3	7.6	6.5	7.0	5.6	4.6	4.3
	E <sub>2</sub>	8.3	8.7	9.1	6.2	8.8	6.1	6.0	5.1
		7.9	7.0	9.3	6.6	8.9	6.1	5.5	5.1
	E <sub>3</sub>	7.2	6.0	8.2	6.5	8.0	6.3	5.4	4.8
		7.9	6.2	8.2	6.7	7.8	6.3	5.5	5.0
18	E <sub>1</sub>	2.2	1.3	2.6	1.8	2.3	2.1	1.1	2.2
		1.9	1.5	2.3	2.0	2.5	2.3	1.1	2.0
	E <sub>2</sub>	1.2	1.9	1.5	1.7	1.3	1.7	1.7	1.4
		1.2	1.7	1.6	1.9	1.4	1.7	1.6	1.6
	E <sub>3</sub>	1.5	1.8	1.4	1.6	1.7	1.7	1.4	1.3
		1.4	1.9	1.6	2.0	1.5	1.5	1.2	1.2

IAA-pretreatment for first four hours

Growth in % per hour

		Conc. <i>gm/ml</i>				IAA			
		0				$10^{-10}$		$10^{-9}$	
0 ↓ 4	E <sub>1</sub>	5.25	5.5	5.75	7.2	6.25	7.0	5.5	5.75
		6.25	6.2	5.5	5.2	5.25	5.75	5.75	5.25
	E <sub>2</sub>	7	6.0	5.75	5.75	6.75	5.0	6.25	4.5
		7.25	5.5	7.5	5.25	7.5	4.5	7.25	5.25
	E <sub>3</sub>	6.25	5.2	6.25	6.0	7.5	7.0	6.25	6.25
		6.2	6.7	7.75	6.5	7.0	7.25	6.0	6.0

Subsequent to IAA-pretreatment, washed, and  
N.M.S.P. treated

Growth in % per hour

		I <sub>o</sub>				I <sub>p</sub>			
		N <sub>0</sub>	N <sub>5</sub>	N <sub>0</sub>	N <sub>4</sub>	N <sub>5I10</sub>	N <sub>5I9</sub>	N <sub>4I10</sub>	N <sub>4I9</sub>
0 ↓ 3	E <sub>1</sub>	6.3	7.0	7.6	9.0	6.6	7.0	8.0	7.3
		5.6	7.3	7.3	8.3	8.0	8.3	7.6	8.0
	E <sub>2</sub>	9.0	9.3	8.1	9.0	9.0	10.0	8.6	8.3
		7.6	9.6	8.0	9.0	9.6	9.6	8.0	8.0
	E <sub>3</sub>	5.6	6.3	8.0	6.0	6.0	6.0	5.3	5.0
		5.0	6.8	7.0	6.0	6.0	6.6	6.0	5.3
3 ↓ 20	E <sub>1</sub>	8.2	10.4	7.7	8.2	10.0	10.0	8.3	8.0
		8.7	10.3	7.2	8.0	10.0	9.9	8.0	8.0
	E <sub>2</sub>	9.2	10.8	8.0	8.4	10.7	10.5	8.0	8.0
		9.4	10.1	7.3	8.5	10.3	9.5	9.0	8.4
	E <sub>3</sub>	8.7	10.0	7.7	10.0	9.0	9.3	9.0	8.0
		9.0	10.0	7.8	9.0	10.0	9.0	9.0	8.2
20 ↓ 44	E <sub>1</sub>	1.8	1.8	1.5	1.5	2.0	1.9	1.0	1.1
		1.8	1.8	1.6	1.7	1.8	1.6	1.1	1.2
	E <sub>2</sub>	.9	1.2	1.6	1.3	.9	.9	1.0	1.2
		1.0	1.0	1.3	1.4	.9	1.4	1.3	1.2
	E <sub>3</sub>	1.2	1.1	1.7	1.6	1.0	.8	1.0	1.0
		1.3	1.0	1.6	1.0	1.0	1.0	.9	1.0



## Interaction of N.M.S.P.-IAA in light and dark

Ratios of the treated samples to dark control

		Dark								Light							
		N <sub>0</sub>	N <sub>8</sub>	N <sub>0</sub>	N <sub>7</sub>	N <sub>0</sub>	N <sub>6</sub>	N <sub>0</sub>	N <sub>5</sub>	N <sub>0</sub>	N <sub>8</sub>	N <sub>0</sub>	N <sub>7</sub>	N <sub>0</sub>	N <sub>6</sub>	N <sub>0</sub>	N <sub>5</sub>
I <sub>0</sub>	E1	101.1	103.7	100.4	114.5	97.8	115.3	100.9	134.0	106.7	111.8	113.2	122.5	106.0	113.9	110.0	153.2
		98.9	101.1	99.6	117.6	102.2	117.6	99.1	144.0	105.4	110.1	108.8	128.2	101.9	116.2	108.0	150.0
	E2	99.1	105.6	99.1	109.9	100.7	111.7	100.0	119.5	110.6	115.0	102.6	107.7	103.0	123.5	105.1	130.5
		100.9	103.7	100.9	105.6	99.3	114.4	100.0	124.3	108.8	106.9	104.7	107.7	103.4	121.7	106.2	132.7
I <sub>11</sub>	E1	104.5	96.8	112.3	99.6	111.1	112.9	113.6	121.4	104.3	107.1	111.8	110.3	106.0	115.3	111.3	132.7
		104.3	93.8	114.3	99.7	112.9	113.0	116.8	118.2	106.7	101.9	107.9	117.6	101.9	113.0	110.0	129.5
	E2	110.4	108.1	107.7	104.1	105.3	114.4	108.4	113.7	115.0	102.7	109.9	104.6	106.1	109.8	112.4	119.5
		108.8	108.1	104.3	102.2	109.8	107.5	108.4	112.8	112.5	104.7	103.9	98.7	108.9	111.8	104.0	120.3
I <sub>10</sub>	E1	93.3	101.9	97.0	106.2	101.4	119.9	100.0	125.0	111.8	106.2	116.7	119.4	107.9	110.6	117.1	135.4
		93.8	98.5	107.9	110.1	99.1	111.2	99.1	119.1	113.9	107.5	112.3	113.2	104.6	115.3	117.3	139.5
	E2	100.0	104.5	94.6	101.9	93.8	112.1	99.6	117.3	105.0	100.0	104.7	106.9	109.8	115.6	106.9	131.8
		102.0	100.4	100.2	99.1	94.7	106.2	101.8	116.8	108.1	101.0	105.6	100.2	105.3	114.4	110.0	127.4

## IAA - P.C.I.B. Interaction

Growth (Fresh weight) in percentage / hour

		I <sub>0</sub>								I <sub>11</sub>								I <sub>10</sub>							
		P <sub>0</sub>	P <sub>7</sub>	P <sub>0</sub>	P <sub>6</sub>	P <sub>0</sub>	P <sub>3X6</sub>	P <sub>0</sub>	P <sub>5</sub>	P <sub>0</sub>	P <sub>7</sub>	P <sub>0</sub>	P <sub>6</sub>	P <sub>0</sub>	P <sub>3X6</sub>	P <sub>0</sub>	P <sub>5</sub>	P <sub>0</sub>	P <sub>7</sub>	P <sub>0</sub>	P <sub>6</sub>	P <sub>0</sub>	P <sub>3X6</sub>	P <sub>0</sub>	P <sub>5</sub>
0 ↓ 5	E1	6.2	6.4	5.6	6.2	5.0	5.2	5.2	4.4	7.2	5.6	7.4	6.6	7.0	4.8	6.8	4.6	6.6	5.2	6.6	5.2	5.8	5.0	6.4	3.6
		5.6	6.4	6.0	5.6	4.6	5.6	4.8	4.4	7.4	5.8	7.0	5.6	6.6	4.4	6.0	4.2	6.4	5.4	6.6	4.8	5.8	4.4	6.4	3.4
	E2	5.8	6.8	6.4	6.2	6.4	7.2	6.6	3.6	7.6	5.6	8.0	5.8	8.4	6.8	8.8	4.8	7.4	5.2	8.	5.0	8.	6.2	7.6	4.4
		8.0	7.0	5.8	6.6	6.0	7.4	6.4	4.2	8.0	5.6	8.5	6.4	7.6	7.2	8.4	4.4	7.2	5.4	7.2	5.8	7.	7.2	7.2	4.0
	E3	5.0	5.6	6.4	6.4	6.2	6.6	7.0	5.6	7.6	5.6	8.0	5.8	7.6	6.4	8.8	6.6	6.4	5.6	9.0	5.8	6.6	5.8	8.8	6.0
		5.0	4.0	6.6	6.6	5.8	7.2	7.0	5.8	6.8	4.8	9.6	5.8	7.6	6.0	10.0	7.2	5.6	5.6	9.6	6.0	6.8	5.6	8.9	6.2
5 ↓ 24	E1	8.1	7.2	8.0	7.6	8.2	7.6	7.9	7.6	8.3	7.5	7.8	7.5	8.1	8.1	8.1	7.5	7.2	7.1	7.4	7.4	7.4	7.4	8.0	7.9
		7.9	7.3	8.1	7.7	8.2	7.8	7.7	7.3	7.9	7.6	7.9	7.6	7.6	7.8	8.4	7.9	7.5	7.1	7.7	7.5	7.5	7.5	7.8	7.5
	E2	7.5	7.4	7.6	7.5	8.2	8.4	8.2	9.0	7.9	7.8	7.7	7.7	8.1	8.2	8.4	8.5	7.3	7.1	7.2	7.6	8.1	8.1	8.0	8.4
		7.7	7.3	8.0	8.0	7.9	8.4	7.9	8.6	7.6	7.6	7.7	7.5	8.3	7.8	8.6	8.6	7.5	7.2	7.3	7.6	8.4	7.8	7.9	8.4
	E3	7.5	7.5	8.0	7.8	7.7	7.6	8.4	9.0	7.2	6.9	7.6	7.4	7.9	7.7	8.4	8.2	6.9	6.7	7.9	7.8	7.1	7.1	7.9	8.1
		7.4	7.5	8.0	8.0	7.9	8.1	8.2	8.9	7.2	6.9	7.4	8.0	7.3	7.2	7.9	8.2	7.2	6.8	7.5	7.8	8.3	6.9	7.7	8.6
24 ↓ 48	E1	1.5	1.5	1.2	1.5	2.4	2.8	1.5	1.3	2.0	1.3	1.3	1.5	2.7	2.0	1.6	2.1	1.5	1.7	1.4	1.3	2.3	2.0	1.5	1.3
		1.5	1.7	1.1	1.5	2.7	2.3	1.8	1.5	1.5	1.1	1.3	1.5	3.1	2.3	1.7	1.8	1.4	1.4	1.1	1.5	2.4	2.5	1.6	1.7
	E2	2.4	1.9	1.6	2.7	2.0	1.8	1.8	1.6	1.7	1.8	2.4	2.1	2.3	1.5	2.0	2.0	2.0	2.5	2.0	2.1	2.1	1.6	2.2	1.5
		2.2	2.2	1.5	2.6	2.0	1.7	1.7	2.1	2.2	2.2	1.9	2.2	2.3	1.9	1.8	1.9	2.2	2.3	2.5	2.4	2.3	1.7	2.2	1.4
	E3	2.	2.3	1.7	1.9	1.8	1.9	1.5	1.0	2.2	2.4	1.9	1.8	2.1	1.3	1.6	1.0	2.2	1.9	1.7	1.4	2.2	1.9	1.9	1.3
		1.9	2.4	1.9	1.8	2.0	1.5	1.8	1.1	2.8	2.3	1.9	1.6	2.3	1.7	1.6	1.0	2.1	1.8	1.5	2.3	2.2	2.2	1.7	1.1



Interaction of P.C.I.B. and IAA  $10^{-8}$  (inhibitory concentration)

Growth in % per hour

		I <sub>0</sub>						I <sub>8</sub>					
		P <sub>0</sub>	P <sub>7</sub>	P <sub>0</sub>	P <sub>6</sub>	P <sub>0</sub>	P <sub>3X6</sub>	P <sub>0</sub>	P <sub>7</sub>	P <sub>0</sub>	P <sub>6</sub>	P <sub>0</sub>	P <sub>3X6</sub>
0 ↓ 4	E1	5.0	6.5	4.7	4.2	7.0	6.0	2.7	2.7	2.5	3.0	3.7	4.0
		5.5	6.0	5.0	4.7	6.0	6.0	2.5	2.7	2.7	3.0	3.5	4.5
	E2	6.0	6.0	5.0	6.0	4.0	4.0	3.0	3.2	3.7	3.5	3.3	3.3
		5.0	6.2	4.7	5.0	4.5	4.2	3.5	3.5	3.5	4.3	3.3	3.6
	E3	5.3	5.2	5.2	5.2	5.5	5.0	4.5	3.5	2.5	3.0	2.5	3.6
		6.2	5.2	6.0	5.0	6.0	5.0	4.6	3.7	3.0	3.2	2.6	3.6
4 ↓ 7	E1	6.6	7.3	7.0	8.3	10.0	9.6	5.0	4.7	4.3	4.3	5.0	7.0
		7.3	8.3	8.3	8.0	8.6	9.3	4.3	4.3	4.3	3.6	4.0	6.0
	E2	6.0	6.6	9.6	9.0	9.0	9.0	3.0	3.6	4.0	4.3	3.3	4.3
		7.6	6.3	7.6	10.3	7.3	8.3	3.3	4.0	4.0	4.0	4.0	3.3
	E3	7.3	8.0	7.6	7.0	6.3	6.6	3.6	3.2	4.6	4.3	3.3	3.3
		8.0	7.0	6.3	6.6	6.3	7.0	3.0	3.5	4.0	4.3	3.0	3.6
7 ↓ 24	E1	9.3	9.2	8.0	8.2	10.0	10.0	8.3	8.4	6.3	8.6	8.8	9.4
		9.0	9.2	7.5	8.2	10.3	10.0	8.8	8.3	6.2	8.3	9.0	9.8
	E2	9.8	8.0	7.8	7.5	7.7	8.0	8.1	8.8	7.2	7.7	7.3	7.8
		9.0	8.0	7.5	8.3	8.4	8.2	8.8	8.2	7.4	7.5	7.0	7.0
	E3	10.0	8.0	8.1	8.1	9.4	8.6	9.0	8.5	6.0	7.6	7.3	7.0
		10.0	8.3	8.3	8.1	8.6	8.3	8.0	8.4	6.2	6.7	7.8	7.3
24 ↓ 48	E1	1.1	1.3	2.6	2.8	1.2	1.1	1.2	1.4	2.4	2.5	1.0	.9
		1.3	1.4	2.6	2.7	1.0	1.0	.9	1.1	2.4	2.5	1.0	.9
	E2	1.0	2.3	2.0	1.6	1.7	1.5	1.3	1.6	1.4	1.0	1.0	1.0
		1.6	2.0	1.9	2.0	1.2	1.7	1.0	1.6	.9	1.4	1.0	1.1
	E3	1.0	1.0	1.3	1.2	1.4	1.6	1.0	1.4	1.0	1.5	1.3	1.5
		1.2	1.7	1.2	1.3	1.3	1.8	1.0	1.0	1.0	1.6	1.2	1.3

IAA-C.N.B. Interaction

Growth percentage per hour.

(24)

		I <sub>D</sub>								I <sub>11</sub>								I <sub>10</sub>							
		B <sub>0</sub>	B <sub>7</sub>	B <sub>0</sub>	B <sub>6</sub>	B <sub>0</sub>	B <sub>5</sub>	B <sub>0</sub>	B <sub>4</sub>	B <sub>0</sub>	B <sub>7</sub>	B <sub>0</sub>	B <sub>6</sub>	B <sub>0</sub>	B <sub>5</sub>	B <sub>0</sub>	B <sub>4</sub>	B <sub>0</sub>	B <sub>7</sub>	B <sub>0</sub>	B <sub>6</sub>	B <sub>0</sub>	B <sub>5</sub>	B <sub>0</sub>	B <sub>4</sub>
0	E1	5	6.7	5.0	5.0	5.0	8.5	4.7	4.3	7.5	5.7	6.0	5.7	8.7	6.5	5.5	4.3	5.7	5.7	6.7	4.8	7.5	8.0	5.7	4.0
		4	5.7	5.2	6.0	5.2	8.5	4.5	4.5	6.2	7.0	6.2	5.0	8.2	7.7	5.0	5.2	7.5	5.5	6.2	4.5	8.2	7.7	5.2	4.0
	E2	6.0	6.5	5.5	7.5	5.0	7.0	4.5	4.7	8.0	7.2	7.2	8.0	6.2	6.5	7.0	4.0	6.2	5.7	7.0	6.7	6.5	5.5	5.0	4.0
		6.5	6.7	6.2	8.	5.0	7.5	4.0	4.7	8.5	6.7	7.5	8.0	6.5	5.7	6.0	4.2	7.5	5.7	6.2	7.5	5.7	5.0	5.7	4.7
4	E3	5	6.0	6.5	8.	4.3	6.0	5.2	5.5	7.2	5.2	8.7	7.0	5.7	5.0	6.5	6.0	7.2	5.7	8.0	6.2	6.2	5.5	6.2	5.2
		5.7	6.2	6.0	7.7	4.5	6.0	5.0	6.0	6.2	5.2	8.5	6.2	6.2	5.7	6.5	6.0	6.2	6.5	7.5	7.5	6.0	6.2	7.2	5.7
	E1	6.3	7.6	5.3	7.3	7.3	8.7	7.6	8.3	8.6	6.3	6.3	7.0	8.3	8.0	8.0	6	7.0	8.5	8.3	7.6	7.0	8.0	8.6	7.5
		6.0	7.6	6.3	6.6	7.6	7.8	7.0	7.3	7.3	7.3	7.6	6.6	8.3	7.7	10.0	7	7.3	5.3	5.0	6.6	7.0	7.3	8.0	7.6
7	E2	5.3	7.3	7.3	9.3	6.3	8	7.3	7.0	5.6	7.0	8.6	9.3	6.0	7.0	8.0	6.0	6.0	5.6	10.0	8.3	6.3	7.0	7.3	7.0
		5.0	7.0	8.3	9.0	5.0	8	7.0	8.0	6.0	7.0	10.0	7.0	6.0	7.0	7.6	5.6	5.0	6.0	9.0	8.6	5.6	6.3	7.3	6.3
	E3	6.6	7.3	5.3	5	6.3	10	7.0	7.6	6.7	6.3	6.3	5.3	7.6	8.6	8.0	8.0	7.0	6.6	5.0	5.6	7.3	8.3	7.6	6.3
		6.0	7.6	6.3	6	6.6	10	6.3	8.0	8.7	5.7	7.0	5.3	8.0	9.0	9.0	7.6	7.0	5.3	6.0	6.0	8.6	7.3	7.1	7.0
24	E1	8.6	8.0	7.8	8.4	8.4	9.5	7.8	8.0	8.5	8.4	8.2	8.4	8.8	8.7	8.0	7.8	8.5	8.6	8.3	7.5	8.5	8.6	7.2	6.8
		8.4	7.9	8.5	8.7	8.1	9.8	7.7	7.4	8.4	8.5	8.3	8.4	9.0	8.9	7.5	7.0	8.4	7.7	8.6	7.2	8.8	8.0	7.6	6.8
	E2	8.8	8.2	8.0	8.0	8.1	9.0	7.1	7.5	9.0	8.2	8.0	8.2	8.4	8.4	7.4	7.7	9.2	7.7	7.8	7.3	8.2	8.3	7.3	7.0
		8.7	8.5	7.9	8.8	8.5	8.6	7.3	7.3	9.7	7.8	8.0	8.0	8.4	9.0	7.5	7.5	8.8	8.1	7.6	7.3	8.0	8.5	7.3	6.7
48	E3	8.2	7.2	9.0	8.8	8.1	8.2	7.8	8.2	8.6	7.7	9.2	9.0	8.0	8.3	8.1	7.4	8.5	8.1	9.0	8.3	8.0	7.3	7.3	6.7
		8.0	7.3	8.9	9.0	8.0	8.4	7.9	7.3	8.5	7.8	9.2	8.4	8.8	8.0	7.8	7.8	7.9	7.4	8.7	8.0	7.8	7.6	7.4	6.9
	E1	1.4	1.5	1.4	1.5	.9	.6	1.6	1.0	1.1	1.7	1.3	1.4	1.1	1.1	1.8	1.4	1.5	1.3	1.3	1.4	.9	.7	1.7	1.2
		1.3	1.5	1.0	1.3	.9	.8	1.4	1.1	1.5	1.4	1.3	1.3	1.2	.7	2.8	1.8	1.0	1.6	1.4	1.5	1.0	1.0	1.5	1.3
	E2	1.2	1.5	1.4	1.3	1.5	1.5	1.5	1.0	1.0	1.5	1.2	1.0	1.7	1.4	1.8	1.0	.9	1.6	1.1	1.2	1.5	1.0	1.7	1.5
		1.3	1.6	1.1	1.2	1.4	1.7	1.5	.8	1.1	1.6	1.3	1.1	1.6	1.1	1.7	1.4	1.3	1.3	1.3	1.1	1.5	1.0	1.9	1.5
	E3	1.2	1.4	1.4	1.7	1.3	1.3	1.0	.6	1.2	.9	1.6	1.6	1.4	1.1	1.0	.7	.8	.8	1.5	1.6	1.5	1.4	1.3	.8
		1.3	1.6	1.4	1.7	1.3	1.4	1.1	.9	1.2	1.0	1.5	1.3	2.0	1.2	1.5	.6	1.3	1.0	1.8	1.6	1.4	1.3	1.3	1.0

B.



N.M.S.A.-IAA Interaction (sub-optimal concentrations)

Growth in % per hour

		I <sub>0</sub>		I <sub>2X12</sub>	
		N <sub>0</sub>	N <sub>2X6</sub>	N <sub>0</sub>	N <sub>2X6</sub>
0 ↓ 4	E <sub>1</sub>	6.5	9.2	7.7	10.0
		6.5	10.0	8.0	8.5
	E <sub>2</sub>	6.7	8.5	7.2	8.0
		7.2	7.7	7.2	7.0
	E <sub>3</sub>	7.0	9.5	7.0	9.2
		7.2	10.0	9.0	10.0
4 ↓ 7	E <sub>1</sub>	7.3	8.6	7.6	7.5
		7.6	8.6	7.6	8.0
	E <sub>2</sub>	5.6	9.6	6.6	8.0
		6.3	9.7	6.3	7.8
	E <sub>3</sub>	9.0	10.0	9.0	10.0
		9.3	11.3	9.1	9.0
7 ↓ 24	E <sub>1</sub>	8.0	9.5	8.3	9.3
		8.6	9.0	8.8	9.0
	E <sub>2</sub>	8.4	9.0	8.8	9.3
		8.8	9.4	8.5	9.4
	E <sub>3</sub>	8.7	9.0	8.8	9.0
		8.7	9.2	8.6	9.0
24 ↓ 48	E <sub>1</sub>	.8	.8	1.0	.5
		.6	.8	.6	.6
	E <sub>2</sub>	.9	1.3	.9	1.2
		.9	.8	1.1	.8
	E <sub>3</sub>	.5	.8	.6	.8
		.6	.7	.8	.7

N.M.S.A.-C.N.B. Interaction

(26)

Growth in percentage per hour

		B <sub>0</sub>																B <sub>5</sub>																B <sub>8</sub>																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																								
		N <sub>0</sub>				N <sub>1</sub>				N <sub>2</sub>				N <sub>3</sub>				N <sub>4</sub>				N <sub>5</sub>				N <sub>6</sub>				N <sub>7</sub>				N <sub>8</sub>				N <sub>9</sub>				N <sub>0</sub>				N <sub>1</sub>				N <sub>2</sub>				N <sub>3</sub>				N <sub>4</sub>				N <sub>5</sub>				N <sub>6</sub>				N <sub>7</sub>				N <sub>8</sub>				N <sub>9</sub>																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																												
0	E1	4.5	4.5	5.7	10	4.7	7.2	6.0	6.5	4.0	4.5	6.2	9.7	4.7	4.7	6.2	4.0	5.0	4.5	9.2	10	7.5	6.7	6.2	5.2	4.2	5.0	6.0	6.8	6.2	4.7	4.2	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4.5	4.2	4

B<sub>0</sub> = 80 C.N.B.  
B<sub>5</sub> = 1 (p.p.m.) C.N.B.  
B<sub>8</sub> = 10 " " " "

B<sub>4</sub> = 100 p.p.m. C.N.B.

N<sub>0</sub> = N<sub>0</sub> N.M.S.A.  
N<sub>5</sub> = 1 (p.p.m.) N.M.S.A.  
N<sub>8</sub> = 10 p.p.m. N.M.S.A.

N<sub>0</sub> = 50 p.p.m. N.M.S.A.  
N<sub>4</sub> = 10<sup>2</sup> " " " "



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P.C.I.B.-N.M.S.P. Interaction

Growth in % per hour.

		No		N5		No		N4	
		P <sub>0</sub>	P <sub>6</sub>	P <sub>0</sub>	P <sub>6</sub>	P <sub>0</sub>	P <sub>6</sub>	P <sub>0</sub>	P <sub>6</sub>
0 ↑ 4 ↓	E <sub>1</sub>	5.2	6.7	6.0	7.2	6.0	5.0	4.5	5.0
		5.5	6.7	5.7	7.2	6.5	4.7	4.5	4.2
		5.0	5.7	7.5	8.0	7.0	5.7	5.0	5.5
	E <sub>2</sub>	4.0	5.0	4.5	4.0	4.0	4.2	3.5	2.5
		3.8	4.0	5.0	5.2	4.5	5.2	4.2	3.0
		4.7	4.7	6.2	5.2	4.5	4.0	3.5	2.5
	E <sub>3</sub>	4.2	3.0	5.5	4.0	4.7	5.2	2.5	2.7
		5.2	3.0	5.0	4.2	5.5	7.0	4.0	3.5
		4.0	5.0	5.0	4.5	5.7	5.2	3.3	2.7
4 ↑ 7 ↓	E <sub>1</sub>	3.0	5.6	6.3	5.0	6.3	5.3	2.0	2.0
		4.0	5.6	7.1	4.3	4.3	4.0	2.0	2.0
		6.0	5.0	5.7	5.3	4.0	5.3	3.0	3.3
	E <sub>2</sub>	7.0	8.3	9.3	7.3	5.3	6.3	3.7	3.7
		8.6	8.3	8.0	7.6	6.3	6.7	3.7	3.3
		7.6	7.7	9.3	7.0	5.0	6.3	3.7	4.3
	E <sub>3</sub>	3.0	5.0	7.3	4.0	5.6	5.7	4.7	2.0
		3.0	6.0	6.0	5.0	6.3	5.2	2.3	2.3
		4.6	5.0	7.3	6.0	7.3	5.6	2.3	4.0
7 ↑ 24 ↓	E <sub>1</sub>	8.8	8.6	10.6	10.8	7.5	7.8	6.5	7.1
		9.4	9.1	11.1	8.7	7.4	7.3	6.5	6.5
		9.5	8.2	10.8	9.5	7.3	7.7	6.7	6.7
	E <sub>2</sub>	7.8	7.2	9.2	9.2	9.0	8.2	6.4	6.2
		8.2	7.6	7.5	8.9	8.7	8.0	5.8	6.0
		8.0	8.1	8.6	9.0	8.3	7.6	6.0	5.7
	E <sub>3</sub>	9.0	7.6	9.9	9.6	8.2	8.1	7.8	7.4
		9.5	7.6	10.2	10.4	8.0	8.7	7.4	7.4
		8.2	7.9	9.9	9.8	8.3	7.9	8.0	6.6
24 ↑ 48 ↓	E <sub>1</sub>	2.9	2.9	2.5	3.0	2.5	2.8	3.1	1.7
		2.4	2.8	3.0	3.3	2.1	2.5	2.8	1.9
		2.7	3.4	2.6	2.9	2.2	3.0	2.8	1.7
	E <sub>2</sub>	2.1	2.1	3.1	3.2	.9	1.4	2.0	1.6
		2.7	1.9	3.1	1.6	1.0	1.6	1.8	1.5
		2.9	2.0	3.0	1.7	1.5	1.5	1.9	1.0
	E <sub>3</sub>	1.9	2.8	2.3	1.6	1.0	2.0	1.2	1.2
		1.9	2.3	2.4	1.3	1.0	1.0	1.0	.8
		2.0	2.2	2.2	1.3	0.9	2.0	.9	1.4